ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

DISSERTATION

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CERTIFICATE

This is to certify that the dissertation entitled "**ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS"** presented here is the original work done by **Dr.S.Rajesh**, postgraduate in the Department of Rheumatology, Madras Medical College & Government General Hospital, Chennai-600003, in partial fulfillment of the University rules and regulations for the award of **D.M BRANCH IX –RHEUMATOLOGY,** under my guidance and supervision during the academic period from 2007- 2010.

Dr. J. Mohanasundaram MD, Ph D, D N B.,
Dean
Madras Medical College &
Government General Hospital,
Chennai -3

Dr. R.Porkodi,MD,DM.,
Professor & Head,
Dept of Rheumatology,
Madras Medical College &
Govt General Hospital, Ch-3

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INTRODUCTION

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Systemic lupus erythematosus (SLE) is an autoimmune disease, with a wide range of clinical manifestations. In 1976, **Urowitz *et al.* (1)** postulated a bimodal mortality pattern in patients with this disease: in the first part of evolution, mortality is due to severe infections or to disease activity, but after 5 years of SLE course, mortality is caused by the accelerated atherosclerosis and its consequences. With the constantly increasing drugs available in the therapeutic armamentarium, even though the early mortality has been brought under control, the late mortality and morbidity associated with SLE remains at high levels. During the last 3 decades, there have been several studies on atherosclerosis in SLE. It has been proved that atherosclerosis has a high incidence among young women with SLE. These patients have a high prevalence of coronary artery disease and an incidence of myocardial infarction up to 50 times higher than age-matched healthy subjects. This high incidence of atherosclerosis in SLE cannot be attributed only to traditional risk factors **(2, 3)**.

Endothelial function is thought to be an important factor in the pathogenesis of atherosclerosis, hypertension and heart failure. In healthy subjects, endothelium is more than a physical barrier and has several functions, like: a) continuous regulation of vascular tone, b) leucocytes adhesion, c) maintenance of the balance between thrombotic and anticoagulant properties of the blood **(4)**. When these functions of the endothelium are affected, endothelial dysfunction appears.

Endothelial dysfunction is considered as the first step in the atherogenetic process. Endothelial dysfunction in SLE is produced by the clustering of traditional risk factors, adverse effects of treatment and SLE itself as an independent risk factor **(5, 6)**. Systemic inflammation, autoantibodies directed to double stranded DNA (dsDNA), ribonucleoproteins (nRNP), endothelial cells, phospholipids, circulating immune complexes, activated complement products, lupus nephropathy and dyslipidemia represent some factors related to SLE which contribute to appearance of endothelial dysfunction **(7,8)**. In the 1990s, high-frequency ultrasonographic imaging of the brachial artery to assess endothelium-dependent flow-mediated vasodilatation (FMD) was developed. The technique provokes the release of nitric oxide, resulting in vasodilatation that can be quantitated as an index of vasomotor function. The noninvasive nature of the technique allows repeated measurements over time to study the effectiveness of various interventions that may affect vascular health **(9)**.

Tremendous interest exists in determining the clinical utility of brachial artery FMD. Investigators have hypothesized that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a preclinical marker of cardiovascular disease **(10)**. Several studies suggest that the presence of endothelial dysfunction in the coronary circulation is an independent predictor of cardiovascular disease events **(11, 12)**. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention. Recently, endothelial dysfunction has also been found in patients with systemic vasculitis and has been reversed by administration of immunosuppressive

therapy **(13)**. As endothelial dysfunction may represent an early stage in atherogenesis, it is important to understand the mechanisms of its development in a condition such as SLE. It is also important to determine whether it is associated with other CHD risk factors or early atheroma.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

SLE predominantly occurs in women, with a gender ratio of 9:1. Onset is typically in the age group of 20- 30 yrs. Multiple predisposing factors have been identified. The genetic predisposition is complex, likely involving more than 100 genes. HLA-DR and DQ alleles are associated not just with the risk of developing lupus, but with the kinds of autoantibodies produced. Genes that control apoptosis (programmed cell death) are important in murine lupus models and likely in human lupus as well. The proteins to which the lupus patient mounts an autoantibody response are exposed on nuclear blebs during apoptosis. Genes involved in immune complex clearance (Fc-gamma receptor alleles) may predispose patients to lupus nephritis. Gene expression studies have identified an "interferon signature"—a group of genes regulated by interferon-—in the majority of SLE patients. The genetic predisposition to SLE is not overwhelming. Only 10% of patients have a first-degree relative with SLE, and SLE develops in only 2% of children who have an afflicted parent.

Environmental factors play a role not only in the onset of SLE but also in triggering the "flares" (relapses). The most recognized environmental trigger is ultraviolet light exposure. Ultraviolet (UV) light significantly by UV - B can trigger photosensitive rashes, and more rarely, systemic flares. SLE patients are more likely than controls to have drug allergies, especially to sulfonamide antibiotics. Infection with Epstein-Barr virus has been strongly associated with SLE in a multicase family registry. Hormonal factors are obviously important, given the female predominance of SLE and the usual onset of SLE after puberty. In clinical trials, hormone

replacement therapy has been associated with increased flares in SLE, but oral contraceptives did not. Pregnancy is associated with SLE flares in some, but not all, studies. Elevation of prolactin may be associated with activity of SLE.

The activity of SLE follows several patterns. The classic pattern, the flare pattern, is characterized by a relapsing-remitting course. However, an equal number of SLE patients have a pattern of continuously active disease. Only a minority of patients have long periods of disease quiescence. The antimalarial drug hydroxychloroquine, which is widely used for cutaneous lupus and lupus arthritis, reduces future flares if patients continue to take it. Over half of SLE patients have acquired permanent damage in one or more organ systems. Although damage, such as renal failure and interstitial pulmonary fibrosis, can occur from SLE itself, immunosuppressive therapy also contributes to certain damage. For example, long-term prednisone therapy may cause osteoporotic fractures, osteonecrosis, cataracts and glaucoma.

Survival of SLE patients peaked at about 80% at 10 years after diagnosis in the 1980s. The Centers for Disease Control and Prevention reported in 2002 that mortality in young women had actually increased. The major cause of death in SLE is accelerated atherosclerosis. Although SLE itself can damage the endothelial surface of the coronary arteries, part of the atherosclerotic process results from elevated levels of traditional cardiovascular risk factors, including hypertension, dyslipidemia, obesity, and homocysteine levels. Prednisolone increases the patient's weight, blood pressure, glucose, and lipid levels. SLE nephritis can lead to

hypertension and dyslipidemia. Renal insufficiency can increase serum homocysteine levels.

The description of a bimodal mortality pattern in SLE patients by **Urowitz et al**. in 1976 was an instrumental step toward identifying their increased risk of premature atheromatous cardiovascular disease (CVD). Patients who succumbed to lupus early in the disease course were noted to die most often from complications of disease activity (e.g., organ failure) or therapy. Patients who died later often had quiescent disease, and died from CV events. These findings were substantiated by an autopsy series reported by **Bulkey et al.** in which the majority of a cohort of young women with a mean age of 35 years had significant obstructive atherosclerotic disease of at least one major coronary artery (14). As survival has improved from better means of disease detection and treatment, atherosclerotic CVD has emerged as a significant cause of morbidity and mortality in SLE. The prevalence of cardiovascular and cerebrovascular events in SLE ranges from 6% to 26% **(15, 16, 17, 18, 19, 20, 21, 22, 23 & 24)**. Ischemic cerebrovascular events (stroke/transient ischemic attack) have been reported in 10% to 26%, **(17, 18, 22, 23 & 24)**, whereas MI and angina have been reported in 6% to 11% of SLE patients **(15, 16, 19, 20, 21, 22, 23 & 24)**.

Not surprisingly, autopsy studies as well as the examination of surrogate markers of coronary atherosclerosis in SLE suggest that the prevalence of subclinical atherosclerosis is higher than overt events **(14, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 & 36)**. Autopsy studies reveal atherosclerotic disease of the coronary arteries in SLE in 22% to 54% of cases **(14, 25 & 26)**. Noninvasive studies

including vascular ultrasound, electron beam tomography, and myocardial perfusion studies demonstrate atherosclerotic vascular disease in 17% to 40% of SLE patients **(27, 28, 29, 30, 31, 32, 33, 34, 35 & 36)**. In a cohort of SLE women with a mean age of 44.9 years, 40% were found to have focal carotid plaque as measured by ultrasound **(27)**. Another study of 75 SLE women with a mean age of 38.8 years demonstrated that 28% had coronary artery calcifications **(31)**. **Roman et al**. found carotid plaque in 37% of SLE patients, compared with a prevalence of 15% in age, sex, and race-matched controls **(32)**.

A striking feature of this comorbid condition of SLE is its predilection for premenopausal women. **Ward** evaluated rates of hospitalization for cardiovascular events in a cohort of SLE women compared to a control group **(37)**. Younger SLE women (age 18 to 44) were 2.27 times more likely to be hospitalized with MI and 3.8 times more for congestive heart failure than controls. In the middle-aged women (45 to 64 years), the frequency of hospitalization for heart failure was just 1.39 times higher, and the frequency for MI hospitalization did not differ significantly from that of controls. A study comparing SLE women with age-similar women from the Framingham offspring cohort demonstrated a 50-fold increased risk of MI in the SLE women between 35 and 44 years of age **(38)**. In sharp contrast to women in the general population, where the risk of atherosclerotic CV events is highest after menopause, the mean age at the first event in SLE women was earlier in their life **(39)**.

Traditional risk factors for CVD occur frequently in SLE, both as a consequence of disease activity and treatment. The presence of subclinical inflammation in the general population has been demonstrated to correlate with the development of a number of traditional risk factors, including insulin resistance, visceral adiposity and hypertension **(40, 41 & 42)**. It is possible that the sustained systemic inflammation and immune activation in SLE has similar influences on the development of CV risk factors. The Toronto Risk Factor Study compared 250 SLE patients with 250 controls and found that SLE patients had a higher number of CV risk factors per patient as well as a higher prevalence of diabetes, hypertension, and elevated levels of low-density lipoproteins, triglycerides, and homocysteine **(43)**. Additionally, both **Bruce et al. and Costenbader et al.** have demonstrated that even when CV risk factors are identified in SLE patients, they often are not adequately treated **(44,45)**. Although a large part of CVD risk in SLE is likely a result of a high prevalence of traditional CV risk factors, **Esdaile et al.** demonstrated that the presence of CV risk factors alone does not explain the increased incidence of CV events **(46)**.

Glucocorticoid therapy has been implicated in atherosclerosis in both lupus and nonlupus patients, but it is unclear whether this reflects pro-atherogenic effects of the underlying disease process or adverse metabolic effects associated with steroid use **(47, 48, 49)**.

Antiphospholipid antibodies have been considered as a contributory factor to SLE-associated atherosclerotic disease. In addition to their postulated role in arterial endothelial damage, they have been associated with renal arterial disease (both

thrombotic and stenotic), which may result in hypertension from activation of the renal-angiotensinogen-aldosterone axis from renal hypoperfusion **(50, 51)**. Although cohort studies have failed to identify an association between the presence of antiphospholipid antibodies and surrogate markers of coronary atherosclerosis, there is a strong association between surrogate markers of CVD and CV events with hypertension with in SLE **(22, 52 and 53)**. The importance of this association becomes apparent when more closely evaluated.

Cardiovascular disease and atherosclerosis are a common cause of morbidity and mortality in various SLE cohorts. Autopsy studies from the early 1980s showed severe atherosclerosis in 40% of SLE patients compared with 2% of control subjects, matched for age at the time of death. Analysis of the Swedish Hospital Discharge Register followed by linkage to the Cause of Death Register during the period 1964 to 1995 showed that SLE patients were at increased risk for death as a result of coronary heart disease or stroke (standardized mortality ratio 2.97, 95% confidence interval 2.78 to 3.16). The risk was substantially higher in the younger group of patients (20 to 39 years old; standardized mortality ratio 16, 95% confidence interval 10.4 to 23.6).

Other studies have shown that SLE patients carry an increased risk for myocardial infarction or stroke compared with the healthy population. This risk cannot be fully explained by the traditional cardiovascular disease risk factors. Atherosclerosis—defined by coronary artery calcification or carotid plaque size—also is more common in SLE patients than in healthy controls (e.g., 31% versus 9%, in subjects with an average age of 40; relative risk 9.8, 95% confidence interval 2.5

to 39), even after adjustment for possible confounding factors, and it correlates with disease activity and damage scores.

Pathogenesis of coronary atherosclerosis in SLE:

Immune Complex or Arteritis

Animal models suggest that this contributes to atherosclerosis. Vascular injury, through immune complexes, followed by exposure to atherosclerotic risk factors, can lead to atherosclerosis in animal models. Immunization of rabbits with heat-shock protein 60/65 leads to aortic intima atherosclerosis. Coronary vasculopathy and myocardial infarctions are found in murine lupus models, often in association with anticardiolipin antibody. Immune complexes from lupus sera accelerated uptake of cholesterol by smooth muscle cells. One small study in human SLE suggested that patients treated with corticosteroids had less intimal proliferation in their coronary vessels, suggesting that suppression of arteritis initially might lead to less atherosclerosis later.

Anti-phospholipid Antibodies

Anti-phospholipid antibodies could contribute to coronary artery disease through thrombosis or vasculopathy. The association of anti-phospholipid antibodies with coronary artery disease has been shown in some but not all studies. It has been found that the lupus anticoagulant is associated with angina/myocardial infarction, but not carotid plaque. Lupus anticoagulant was increased in SLE cases with cardiovascular disease vs those without. Anti-phospholipid antibodies may function also as antibodies against oxidized lipoproteins, an additional mechanism (the

"oxidative modification hypothesis") by which they might contribute to atherosclerosis. In one study, anti-oxLDL was higher in SLE cases with cardiovascular disease. However, several studies have failed to find an association of antioxLDL with arterial thrombosis, arterial disease or atherosclerosis. Finally, one of the plasma protein targets of antiphospholipid antibodies, b2 glycoprotein I, may be an important control against atherosclerosis that is perturbed by anti-phospholipid antibodies. Anti-b2 glycoprotein I accelerates uptake of oxLDL in vitro. There is also interest in lysophosphatidycholine, LPC, a high-affinity ligand for G2A, a lymphocyte expressed protein-coupled receptor. Genetic deletion of the receptor results in autoimmunity. LPC is reduced in SLE and anti-LPC has been detected.

Chronic Infection

In the non-SLE patient, there is great interest in chronic infections, including Chlamydia pneumoniae, as potential causes of atherosclerosis. Simple antibiotic regimens could conceivably eliminate these infections and reduce later coronary artery disease. Whether these chronic infections lead to accelerated atherosclerosis in SLE is currently unknown.

Coronary Artery Disease Risk Factors

Multiple studies have now proven that the risk of coronary artery disease (CAD) in SLE cannot be solely explained by traditional cardiovascular risk factors. After controlling for common risk factors at baseline, SLE patients have a relative risk of 10.1 for nonfatal MI. In a study with control subjects matched for traditional cardiovascular risk factors, SLE patients had more carotid atherosclerosis (41 vs

9%) and left ventricular hypertrophy (32 vs 5%). On average, SLE patients with coronary artery disease have one less traditional cardiovascular risk factor than a control patient. Although traditional risk factors cannot explain all atherosclerosis in SLE, they contribute significantly to the process.

Several works have found that routine coronary artery disease risk factors are very frequent in SLE patients. In fact, the average SLE patient in a cohort study has 3 or more of these routine CAD risk factors. Some of these risk factors could be due to SLE. Hypertension, for example, is more prevalent in SLE patients with renal disease. Hypertension is associated with coronary artery disease in SLE, including some, but not all, multivariate analyses.

Hyperlipidemia in SLE has two major patterns. One pattern occurs in active disease, especially in pediatric patients. These patients have low HDL cholesterol and apoprotein A1 with elevated VLDL cholesterol and triglyceride levels. Lahita and colleagues have found a similar dyslipoproteinemia in SLE patients with anti-cardiolipin antibody. However, one group has found that disease activity, rather than anti-cardiolipin, explains the reduction in HDL. There are likely defects in early cholesterol transport and VLDL metabolism associated with active SLE. The second pattern occurs in SLE patients on corticosteroids, with higher levels of triglyceride, cholesterol, and LDL cholesterol. Sustained hypercholesterolemia, rather than baseline, or intermittent elevation, is the most important predictor. A third problem identified is decreased lipolysis and chylomicron remnant removal. Lipoprotein (a) (Lpa) has been identified as a risk factor for atherosclerosis in SLE. SLE patients can make anti-lp (a). Lp (a) may rise with disease flares and be reduced by

corticosteroids. SLE patients with higher lp (a) levels also have more immune complexes containing b2 glycoprotein I.

The major cause of death in lupus nephritis patients is cardiovascular disease. Traditional cardiovascular risk factors, especially hypertension and hyperlipidemia, are increased. Tubulointerstitial lipid deposits can be found. In juvenile-onset SLE, nephrotic range proteinuria is the strongest risk factor for atherosclerosis. In a longitudinal study, three CAD risk factors, weight, cholesterol, and mean arterial pressure, were worsened by prednisone therapy. In the regression model, a 10-mg increase in prednisone led to a 5.5-lb increase in weight and an 8.9-mg% increase in total cholesterol, adjusting for all other factors known to affect these risk factors. Thus, even if the development of these CAD risk factors is directly due to SLE, prednisone treatment increases their levels.

A recent work has focused on a newly identified risk factor for cardiovascular disease, homocysteine. Homocysteine is an amino acid that has a direct toxic effect on endothelium and indirect effects, including induction of a vascular endothelial cell activator, promotion of vascular smooth muscle proliferation, and an inhibitory effect on endothelial cell growth. Hyperhomocysteinaemia has been shown to increase the risk of coronary artery disease, stroke, and carotid artery stenosis in the Physicians' Health Study and the Framingham Heart Study. In the Hopkins Lupus Cohort Study, 15% of the 337 SLE patients had elevated homocysteine. Raised homocysteine levels were significantly associated with stroke and arterial thrombosis. A retrospective study has confirmed this association.

Based on several studies the risk factor frequency can be approximated as follows:

Risk factor Frequency (%)

Family history 41

Hypertension 48

Hypercholesterolemia 56

Obesity (major) 38

Smoking—ever 56

Smoking—current 35

Sedentary lifestyle 70

Diabetes 7

Homocysteine 15

Inflammation plays a major role in the pathogenesis of atherosclerosis and myocardial infarction in the general population. The benefit of statins, for example, is mediated not just by their lipid-lowering effect, but by an anti-inflammatory effect (demonstrated by reduction in C-reactive protein) that likely stabilizes plaques. In the general population, C-reactive protein, IL-6, fibrinogen, and other markers of inflammation are predictive of atherosclerosis. In SLE, C-reactive protein was not predictive in a multivariate model nor in univariate analyses in one lupus cohort. However, in another case-control study, C-reactive protein was increased in SLE cases vs SLE controls. They did not find fibrinogen to be predictive of atherosclerosis. In one study, fibrinogen increased with duration of disease regardless of disease activity, and was increased in SLE patients with thrombosis.

Prolonged corticosteroid therapy could precipitate atherosclerosis indirectly, by increasing the levels of CAD risk factors (hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, obesity, and homocysteinemia) or directly, via vascular injury. Accelerated atherosclerosis has occurred in SLE patients without corticosteroid use, but such cases are very rare. In a 1986 review, Nashel made a strong case that the latter occurs. In animals, corticosteroids and ACTH produce vascular injury, alter vascular connective tissue and worsen experimentally induced atherosclerosis. Patients with Cushing's syndrome, before effective treatment was available, commonly developed accelerated atherosclerosis.

Flow mediated Dilatation:

The capacity of blood vessels to respond to physical and chemical stimuli in the lumen confers the ability to self-regulate tone and to adjust blood flow and distribution in response to changes in the local environment. Many blood vessels respond to an increase in flow, or more precisely shear stress, by dilating. This phenomenon is designated FMD. A principal mediator of FMD is endothelium-derived NO. The precise mechanisms for the acute detection of shear forces and subsequent signal transduction to modulate vasomotor tone are not fully understood. The endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear stress **(54, 55 & 56)**. The effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry (there are no voltage-gated calcium channels in endothelial cells). Calcium activates an enzyme, endothelial nitric oxide synthase (eNOS), and the subsequent generation of NO appears to account for FMD

(57, 58). Indeed, endothelial denudation or treatment with a nitric oxide synthase (NOS) inhibitor abolishes FMD in a variety of arterial vessels. However, it was recently shown that blood vessels from mice genetically engineered to lack the eNOS enzyme (eNOS knockout mice) still respond to shear stress by dilating. In the eNOS knockout mice, FMD seems to be mediated by endothelium-derived prostanoids, as it is blocked by indomethacin **(59)**. Thus, there is some redundancy in the system, and more than one endothelial mediator is capable of acting as the signal between endothelium and smooth muscle. It is unknown whether other mediators, such as the putative endothelium-derived hyperpolarizing factor, can cause FMD if both NO and prostanoids are deficient.

Several mechanisms may underlie the increase in NO in response to changes in shear stress. Very acute changes may be mediated by the increase in intracellular calcium that occurs when ion channels open (see the previous text). Over slightly longer time periods (minutes), shear-stress-induced phosphorylation of eNOS via a serine/threonine protein kinase, Akt/PKB, increases eNOS activity, even at low calcium concentrations, and this may be important to allow continued output of NO **(60, 61)**. In addition, other posttranslational modifications of the enzyme (myristilation or palmitoylation) or interaction with caveolin can affect intracellular localization of the enzyme and thereby alter its function. Over longer time periods (many minutes or hours), eNOS gene transcription is activated, and this can result in continued increases in NO generation if shear stress is maintained at high levels.

To assess endothelial function noninvasively, brachial arteries are scanned with high resolution ultrasound imaging, under baseline conditions (at rest) and during hyperemia induced by inflation and deflation of a sphygmomanometer cuff mostly around the forearm distal to the site to be scanned with ultrasound. The induced shear stress caused by the increased blood flow following transient ischemia induces nitric oxide (NO) release, which in turn causes local arterial vasodilatation**.** Endothelial function, defined as flow mediated dilatation (FMD), is estimated as the percentage increase in vessel diameter from baseline conditions to maximum vessel diameter during hyperemia. Impaired endothelial function of the brachial artery assessed in this manner has been reported in asymptomatic children and adults with elevated cardiovascular risk factors such as smoking **(62)**, hypercholesterolemia **(63)**, hypertension **(64)**, diabetes mellitus **(65)**, and hyperhomocysteinaemia **(66)**. Although the results of these studies are likely to be internally valid, comparison of the FMD values across studies is troublesome. FMD values vary considerably across populations, ranging from –1.9 to 19.2%.

Masoud El-Magadmi et al (67) studied 62 women with SLE (1997 revised criteria) and 38 healthy women. Demographic and risk factor data were collected. In patients, disease activity and treatment-related parameters were also assessed. Endothelial function was assessed by flow-mediated dilation (FMD) in the brachial artery in response to reactive hyperemia. Carotid intima-media thickness (IMT) and the presence of carotid plaques were also assessed in SLE patients. FMD was impaired in SLE patients (median, 3.6%; range, –6.3% to 13.7%; versus median, 6.9%; range, –6.6% to 17.8%, $P<0.01$). Using multiple regression analysis that included all subjects in which they retained all the classic CHD risk factors, they

found that systolic blood pressure (P=0.019) and SLE (P=0.017) were significantly associated with impaired FMD. Within SLE patients, IMT showed a negative correlation with percent FMD (r=–0.37, P<0.01). In stepwise multiple regression of SLE patients only, that also included SLE factors and IMT, IMT alone was independently associated with FMD (P=0.037). Two other studies have reported similar findings. In a study from Sao Paulo, **Lima et al** noted that the mean± SD FMD in SLE was 5.0±5.0% compared with 12.0±6.0% in healthy control subjects. In this study, postmenopausal women and subjects with known CHD risk factors were excluded. Piper et al found in a UK cohort that SLE women had a median FMD of 5.6 (interquartile range [IQR], 3.1% to 7.2%) compared with 8.0% (IQR, 6.3% to 9.3%) in control subjects.

Valdivielso P et al (68) analyzed endothelial function in systemic lupus erythematosus (SLE), and its relationship with disease activity and subclinical arteriosclerosis. They studied a group of 26 patients with SLE and 21 age- and sex-matched controls. None of the patients or controls had had any ischemic event. Data were recorded on medical history, anthropometrics, prior treatment and the lupus activity index (LAI). Endothelial function was quantified by flow-mediated dilatation in the brachial artery. The presence of subclinical arteriosclerosis was assessed by the average intima-media thickness (IMT) on carotid ultrasound. The patients and the controls had a similar degree of carotid IMT (0.58+/-0.08 mm vs. 0.57+/-0.07 mm, NS) and a similar prevalence of carotid plaque (27% vs. 24%, NS). However, the SLE patients had worse endothelial function than the controls (FMD 12.4+/-4.4% vs. 16.9+/-5.5%, p<0.05). This difference remained after adjusting for age, smoking, body mass index, waist circumference, total cholesterol, triglycerides,

HDL cholesterol, apolipoproteins A-1 and B100 and postmenopausal status. A significant association was found in the SLE patients between FMD and LAI (Spearman Rho -0.462, $p<0.05$). SLE-associated endothelial dysfunction was present in patients who have no prior ischemic events and with the same degree of subclinical arteriosclerosis as controls. Moreover, the endothelial dysfunction was significantly associated with the degree of disease activity. There was difference among these studies with regard to the inclusion of disease duration, comorbities & use of immunosuppressants.

Piper M K et al (70) compared thirty-six female SLE patients with 22 healthy age and sex matched controls. Endothelial dependent vasodilatation (EDD) was assessed at the brachial artery in response to shear stress. SLE patients showed significantly impaired endothelial function (median EDD 5.6%, IQR 3.1-7.2%) compared with healthy controls (median EDD 8.0%, IQR 6.3-9.3%; P = 0.001).

Zahra Seyyedbonakdar et al (69) evaluated the prevalence of vascular endothelial dysfunction and its risk factors in SLE women and then to identify its correlation with the disease activity, duration and concomitant conditions in these patients. Eighty four female SLE patients and 18 healthy young female were included in the study. The vascular endothelial function was evaluated via ultrasonographic assessment of the brachial artery diameter to determine flow mediated dilation (FMD). SLE patients had higher prevalence of impaired FMD than healthy subjects (48.8 vs. 5.5 %). But FMD impairment did not have any significant correlation with disease activity, duration, the presence of the anti-dsDNA, anticardiolipin antibodies, antiphospholipid syndrome, hypertension,

hyperlipidemia, diabetes mellitus, hypothyroidism, history of lupus nephropathy, and history of receiving cyclophosphamide pulses.

Elizabeth Turner et al (71) measured flow-mediated dilation of the brachial artery using high resolution ultrasound and the presence or absence of coronary calcification by electron beam computed tomography. Twenty patients (17 female) median age (interquartile range) 42.5 (32.0–47.5) years were studied. The median flow-mediated vasodilatation was 3.6% (1.7%–7.7%). In patients with coronary calcification (n = 6), flow-mediated dilation was 2.1% (−0.42%–3.6%) compared with 4.0% (3.5%–8.3%) in those without (p = 0.12). There was no significant relationship between flow-mediated dilation and markers of disease activity, duration of disease, and cardiovascular risk factors. Lower flow-mediated dilation was associated with duration of corticosteroid therapy.

Lai-Shan Tam et al (72) examined whether acute hyperhomocysteinaemia exacerbates endothelial and platelet dysfunction in patients with SLE. Twelve SLE patients and 15 controls were recruited. Oral methionine was used to achieve acute hyperhomocysteinaemia. Endothelial function was assessed by flow-mediated dilatation (FMD) of the brachial artery; also assessed were the levels of von Willebrand factor (vWF) and plasminogen activator inhibitor type 1 (PAI-1). Platelet activation was assessed by the levels of beta-thromboglobulin (beta-TG), fibrinogen binding, and P-selectin expression using flow cytometry. After oral methionine loading, vWF levels increased significantly, whereas FMD remained unchanged in both groups. PAI-1 increased significantly only in controls. Fibrinogen binding to platelets increased significantly only in SLE patients. Beta-TG remained

unchanged in SLE patients but increased significantly in controls. Platelet P-selectin expression did not change in either group. These results suggest that the prothrombotic tendency after acute hyperhomocysteinaemia is mediated by endothelial dysfunction and platelet activation in patients with SLE and healthy controls.

Parasar Ghosh et al (73) studied Asian Indians with SLE, to find out the prevalence and predictors of carotid intima-medial thickness (IMT) and brachial artery flow-mediated dilatation. Endothelial dysfunction was assessed by FMD in brachial artery and IMT was measured in common carotid artery in SLE patients and healthy controls. Sixty SLE patients (mean age 31±9 years) and 38 healthy controls (mean age 34±6 years) were included. The IMT was higher in SLE patients as compared to controls (0.49±0.08 mm vs. 0.39±0.05 mm, $p<0.0001$). SLE and damage were independent predictors of abnormal IMT. FMD was impaired in SLE patients compared to controls (9.97% vs. 18.97%, $p<0.00001$). None of the classical cardiovascular risk factors were predictors of FMD or abnormal IMT. Their study has shown a good negative correlation between brachial FMD and carotid IMT which have also been shown in other studies **(74).**

Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery (9).

Mary C. Corretti, MD et al has led the report submitted by the International Brachial Artery Reactivity Task Force.

Subject preparation:

Numerous factors affect flow-mediated vascular reactivity, including temperature, food, drugs and sympathetic stimuli, among others. Therefore, subjects should fast for at least 8 to 12 h before the study, and they should be studied in a quiet, temperature-controlled room. All vasoactive medications should be withheld for at least four half-lives, if possible. In addition, subjects should not exercise, should not ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.

Equipment:

Ultrasound systems must be equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler and a high-frequency vascular transducer. A linear array transducer with a minimum frequency of 7 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images with sufficient resolution for subsequent analysis. Image resolution is enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers.

Image acquisition:

The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging.

Endothelium-dependent FMD: To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff is first placed either above the antecubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume. Thereafter, arterial occlusion is created by cuff inflation to suprasystolic pressure. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation.

Studies have variably used either upper arm or forearm cuff occlusion, and there is no consensus as to which technique provides more accurate or precise information. When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm **(75, 76 & 77)**. This may be

due to a greater flow stimulus resulting from recruitment of more resistance vessels or possibly to direct effects of ischemia on the brachial artery. However, upper-arm occlusion is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue. The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 s to 5 min. The change in diameter is similar after 5 and 10 min of occlusion; therefore, the more easily tolerated 5-min occlusion is typically used. Also, FMD may be studied in the radial, axillary and superficial femoral arteries. Notable caveats are that arteries smaller than 2.5 mm in diameter are difficult to measure and vasodilation is generally less difficult to perceive in vessels larger than 5.0 mm in diameter **(78, 79 & 80)**.

Anatomic landmarks:

The diameter of the brachial artery should be measured from cross sectional images in which the lumen-intima interface is visualized on the near (anterior) and far (posterior) walls. These boundaries are best visualized when the angle of insonation is perpendicular. Thus, clear visualization of both the near and far wall lumen-intima boundaries indicates that the imaging plane is bisecting the vessel and diameters measured from these images likely reflect the true diameter. Once the image for analysis is chosen, the boundaries for diameter measurements are identified manually with electronic calipers or automatically using edge-detection software. The variability of the diameter measurement is greatest when it is determined from a point-to-point measurement of a single frame, and least when

there is an average derived from multiple diameter measurements determined along a segment of the vessel.

The diameter measurement along a longitudinal segment of vessel is dependent upon the alignment of the image. Skew occurs when the artery is not completely bisected by the plane of the ultrasound beam. With slight skew, the maximal diameter measured is constant, and thus yields a more accurate measurement. Some edge-detection programs can account for skew from transducer angulation **(79, 81)**.

Timing of FMD:

Flow-mediated vasodilation is an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased shear stress. Increased flow, and thereby increased shear stress, through the brachial artery occurs during post occlusive reactive hyperemia. Several studies have suggested that the maximal increase in diameter occur approximately 60 s after release of the occlusive cuff, or 45 to 60 s after peak reactive hyperemic blood flow **(80, 82)**. The increase in diameter at this time is prevented by the NOS inhibitor NG-monomethyl-L-arginine, indicating that it is an endothelium-dependent process mediated by NO **(83, 84)**. Other measures of vasodilator response include time to maximum response **(85)**, duration of the vasodilator response and the area under the dilation curve.

Characterizing FMD:

Flow-mediated vasodilation is typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter **(86)**.

Evaluating precision of the technique:

Intraobserver and interobserver variability in image acquisition and analysis should be established and periodically reassessed for each condition, including baseline, reactive hyperemia and NTG administration. The highest reproducibility is likely to be shown over a short interval, during which the individual vasodilator response is unlikely to have changed owing to environmental or other influences. This can be accomplished by taking two measurements on the same day within a 10- to 15-min interval, or on separate days in otherwise identical circumstances. Longitudinal studies in which interventions over weeks to months are tested require that reproducibility measurements be performed at longer intervals. The image analysis and measurement of the vasodilator response from repeated studies should be performed by an individual who is blinded as to sequence. Measurement variability is assessed, typically, by a designated core laboratory for multicenter trials, prior to site certification and periodically thereafter to analyze for temporal drifts. Assessment of FMD of the brachial artery in clinical trials has increased because of its seeming ease of use, efficiency and noninvasive nature. Owing to the biological and technical variability of the measurement, several caveats should be considered when planning a clinical trial where FMD is the end point of interest. These include study design, sample size and uniform technique.

Study design:

Recent studies have reported on the effect of pharmacologic or physiologic interventions on FMD of the brachial artery. These include both acute **(87, 88, 89)**

and longer-term intervention trials **(90, 91&92)**. Both parallel-group and crossover designs have been successfully employed.

Sample size.

Typically, significant improvement in FMD can be seen with 20 to 30 patients in a crossover design study and 40 to 60 patients in a parallel-group design study. In studies of this size, the minimal statistically significant improvement that can be detected with intervention is an absolute change in FMD of 1.5% to 2%. The sample size depends greatly on the variance of repeated measurement in the control group in a particular vascular laboratory.

With intervention trials, an important parameter to report is the time-dependent reproducibility of FMD. For example, in the placebo group, the pretreatment and post intervention FMD measures are usually reported, and often are very similar. However, if the mean difference between the two measurements for each patient is quite high, it indicates that the variance of the technique might limit interpretation of the study results. An acceptable reproducibility is a mean difference of 2% to 3% in FMD over time (on a baseline vasodilation of about 10%) **(93)**. This value has not been readily available in published trials.

Methodology:

As discussed above, several techniques have been employed to measure FMD **(94, 95)**. Laboratories should select the method that gives them the most reproducible results, and for multicenter studies, the same scanning protocol should be employed at all sites. For studies employing repeated measurements following

intervention, FMD might change as a result of the intervention. However, FMD could also be affected by a change in the hyperemic stimulus. Therefore, the flow stimulus should be consistent. Otherwise, any change in FMD of the conduit artery may be related to changes in flow (indirectly mediated by changes in the microcirculation) rather than improvement of endothelial function of the conduit vessel per se.

Ultrasound assessment of brachial artery FMD has yielded important information about vascular function in health and disease, yet several new approaches and technological advances have emerged. Most prior studies examined FMD at a single time point, typically 1 min after cuff release. This practice evolved from the observations that the maximal dilator response occurs at approximately 1 min in healthy subjects (96) and that the necessity for manual acquisition and measurement placed a practical limit on the number of image frames that could be analyzed.

Commercially available technology now makes it possible to acquire multiple images of the brachial artery automatically using the ECG signal as a trigger and to measure arterial diameter automatically using computer-based edge-detection techniques. This approach allows investigators to examine the entire time course of brachial dilation in response to reactive hyperemia, the true peak response, the time to peak and the overall duration of FMD as discussed in the previous text. The time course and extent of brachial expansion within a single cardiac cycle, possibly reflecting vessel compliance, can be examined. In the carotid artery,

compliance has been shown to correlate with cardiovascular risk **(97)**. About 70% of the dilation observed 1 min after cuff release is attributable to NO synthesis **(98, 99)**.

AIM

AIM OF THE STUDY

1) To evaluate endothelial function and to assess the extent of dysfunction in newly diagnosed SLE patients by using the measures of flow mediated dilatation of the brachial artery and carotid intima media thickness.

2) To study the correlation of flow mediated dilatation with carotid intima medial thickness.

3) To study the relationship between endothelial dysfunction and clinical characteristics of SLE.

MATERIALS AND METHODS

MATERIALS AND METHODS

Inclusion Criteria:

1) Newly diagnosed SLE patients by ACR criteria of age 16 yrs and above.

Exclusion Criteria:

1) Patients with co morbidities like hypertension, diabetes mellitus and hyperlipidemia.

2) Patients with history of cardiovascular disease (angina, myocardial infarction, congestive cardiac failure).

3) Patients with renal failure (creatinine >3 mg/dl or creatinine clearance <30 ml/min).

4) Patients who were on long term medications like prednisolone, other immunosuppressants & statins prior to our evaluation.

5) Patients with clinical evidence of upper limb vascular insufficiency in the form of pre gangrene or gangrene.

6) Patients with overlap syndrome.

7) Infections in the previous four weeks.

8) Pregnancy or lactation in the previous 6 weeks.

Subjects:

Patients were recruited from the rheumatology outpatient clinic and wards of Government General Hospital, Chennai, during the period February 2009- February 2010. Fifty eligible patients older than 16 years of age were enrolled. They fulfilled at least four classification criteria for systemic lupus erythematosus by 1997 ACR revised criteria, with no known preexisting cardiovascular disease; and were willing to undergo measurement of flow-mediated dilation.

Healthy control subjects were recruited from the clinically normal secretarial and support staff as well as from friends of patients. All subjects gave written informed consent to take part in this study, which was approved by the ethical committee.

Clinical and Laboratory Assessment:

All subjects had a detailed history and examination to look for traditional cardiovascular disease risk factors, vasculitis, Raynaud's phenomenon, and secondary antiphospholipid syndrome. In patients, disease activity and cumulative damage were assessed by the SLE Disease Activity Index (SLEDAI) and the Systemic Lupus International Collaborating Clinics (SLICC) respectively. Laboratory evaluations include complete blood count, ESR (Westergren's method), liver function test, renal function test, fasting blood sugar and lipid profile. Immunological assay were CRP (Latex agglutination method); ANA (CalBiotech Inc ANA Screen ELISA kit); Anti dsDNA (Wampole laboratories dsDNA ELISA test); Anti cardiolipin antibody (CalBiotech cardiolipin IgG & IgM ELISA kit) &

C3, C4 complement levels (Nephelometry). The lupus anticoagulant study was done with the dilute Russell viper venom test & activated partial thromboplastin time; were also assayed.

ELISA results were interpreted as follows:

ANA

< 0.9 – Negative.

0.9- 1.1 - Borderline positive.

> 1.1 - Positive.

Anti dsDNA

< 0.9 - Negative

0.91 – 1.09 – Equivocal

> 1.1 – Positive.

ACL IgG

< 10 GPL– Negative

10- 15GPL – Borderline positive.

15- 80 GPL– Moderate positive.

>80 GPL– High positive.

ACL IgM

< 15 MPL – Negative

15- 20 – Borderline positive

21- 80 – Moderate positive

> 80 – High positive.

Complement Assay (Nephelometry)

Normal range:

C3 - 88 – 201 mg/dl.

C4 – 16 – 47 mg/dl.

All patients had Electrocardiogram, chest X ray, ultrasound abdomen and an Echocardiogram done by a cardiologist.

Assessment of Endothelial Function:

Endothelial function was assessed with high-resolution B-mode Doppler (Siemens Alterces with a 7.5 MHz linear-array transducer **Photo (A))** examination of the brachial artery using the protocol described as in the guidelines cited above **(9)**. We measured flow-mediated dilation (FMD) in response to reactive hyperemia. All subjects were studied between 8 and 11 AM after a12-hour overnight fast. They were asked not to smoke on the morning of study and to avoid alcohol for 48 hours. The brachial artery was scanned 10 cm above the antecubital fossa **(Photo B)**. Distance measured was from anterior to posterior M lines (media-adventitia interface), and every measurement was taken by sonologist blinded to cases and controls. Then, ischemia was induced by inflating the pneumatic cuff to a pressure 50 mmHg above systolic one, in order to obliterate the brachial artery. After 5 minutes, the cuff was deflated and the diameter was measured after 60 seconds post-deflation (Df).

FMD is calculated as follows: 100 %x [(post deflation diameter–resting diameter)/resting diameter]. To assess reproducibility of our technique, we looked at the reliability of reading scans on 2 separate occasions by a single blinded observer. For this, 15 scans from patients or control subjects were chosen at random. The intraclass correlation coefficients for resting diameter and FMD were 0.93 (95% CI, 0.56 to 0.95) and 0.82 (95% CI, 0.30 to 0.97), respectively.

A)

(B)

Carotid Artery Intima -Media Thickness and Plaque:

Patients and control subjects also had the intima-media thickness (IMT) of their carotid artery measured using high-resolution B-mode Doppler (Siemens Alterces with a 3-15 MHz linear-array transducer). The common carotid artery was scanned longitudinally, and the IMT measurement was taken in the proximal part of the common carotid artery, 1 cm proximal to the carotid bulb as the maximum distance between the intima-lumen and adventitia-media interfaces in areas without carotid plaque **(100)**. IMT was determined as the average of 6 measurements, 3 each from the left and right common carotid arteries. This is shown in the **Photo E**.

We also noted the presence or absence of carotid plaques, with plaque being defined using the criteria described by **Li et al (101)**. The intraclass correlation coefficient for IMT measurements, assessed in 15 subjects on 2 separate occasions 2 weeks apart, was 0.92 (95% CI, 0.84 to 1.00).

Statistical Analysis

The statistical analysis was performed using the SPSS (version 17.0).

Results are presented as mean ± SD, except for frequencies, which are expressed as percentages.

Unpaired student's t- test has been used for comparing the FMD, carotid IMT and other features of the study and control group.

$p < 0.05$ was considered statistically significant.

Pearson's test has been used for studying the correlation between flow mediated dilatation and variables.

RESULTS

RESULTS

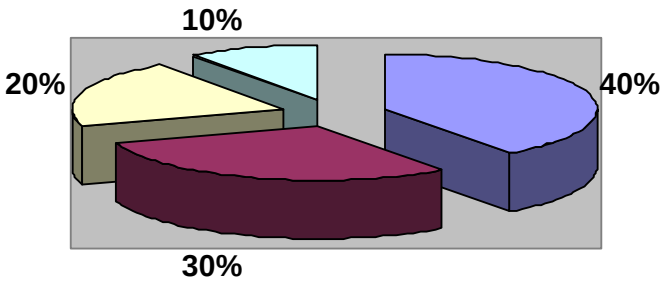
The demographic, clinical and investigation features of the studied groups were as follows:

TABLE 1: Characteristics of the group

Parameter	Patient (n=50)	Control (n=50)	P value
Male/Females	3/47	3/47	1.00
Mean Age (yrs)	23 ± 5.41	23 ± 5.41	1.00
BMI (kg/m2)	19.6 ± 1.77	20 ± 1.71	0.31
Mean duration of symptoms (months)	7.3	0	-
Systolic BP (mmHg)	112 ± 8.03	111 ± 7.7	0.59
Diastolic BP (mmHg)	79.6 ± 5.83	79.2 ± 5.52	0.75
Total Cholesterol (mg/dl)	169 ± 23.4	175 ± 19.7	0.13
Triglycerides (mg/dl)	114 ± 11.9	113 ± 10.2	0.56
HDL (mg/dl)	43.6 ± 3.5	42.9 ± 2.51	0.20
LDL (mg/dl)	108 ± 10.3	105 ± 8.61	0.09

There were 47 females and 3 males, who were age and sex adjusted in the patient and control group. The mean duration of disease according to the onset of symptoms, was 7.3 months with a minimum duration of one month to a maximum of 14 months. There was no statistically significant difference with regard to the blood pressure and fasting lipid profile between both the groups.

Age distribution of the population



Clinical characteristics of the SLE patients:

The patient group had the following clinical features in the proportion given: mucocutaneous – 88% (n=44); constitutional – 86% (n=43); musculoskeletal – 41% (n=82%); serositis – 22% (n=11); hematological – 6% (n=12%); neuropsychiatric – 8% (n=4); renal – 2% (n=1); Raynaud's – 0. The mean SLEDAI score was 11.6.The median SLEDAI score was 11 (range, 3 to 29). The median SLICC was 0 (range 0 to 1). Since we had chosen patients at diagnosis, and by excluding the patients who had been on immunosuppressants prior to our evaluation, we had a relatively naïve population who had not been on specific disease modifying drugs.

On evaluation, the patient population had the following immunological features.

TABLE 2: Immunological profile the patient group was as follows:

Parameter	Patient group (n=50)
ANA	100% (n=50)
Anti dsDNA	78% (n=39)
Low C3/C4 assay	36% (n=18)
ACL IgG/IgM	16% (n=8)
LAC	10% (n=5)

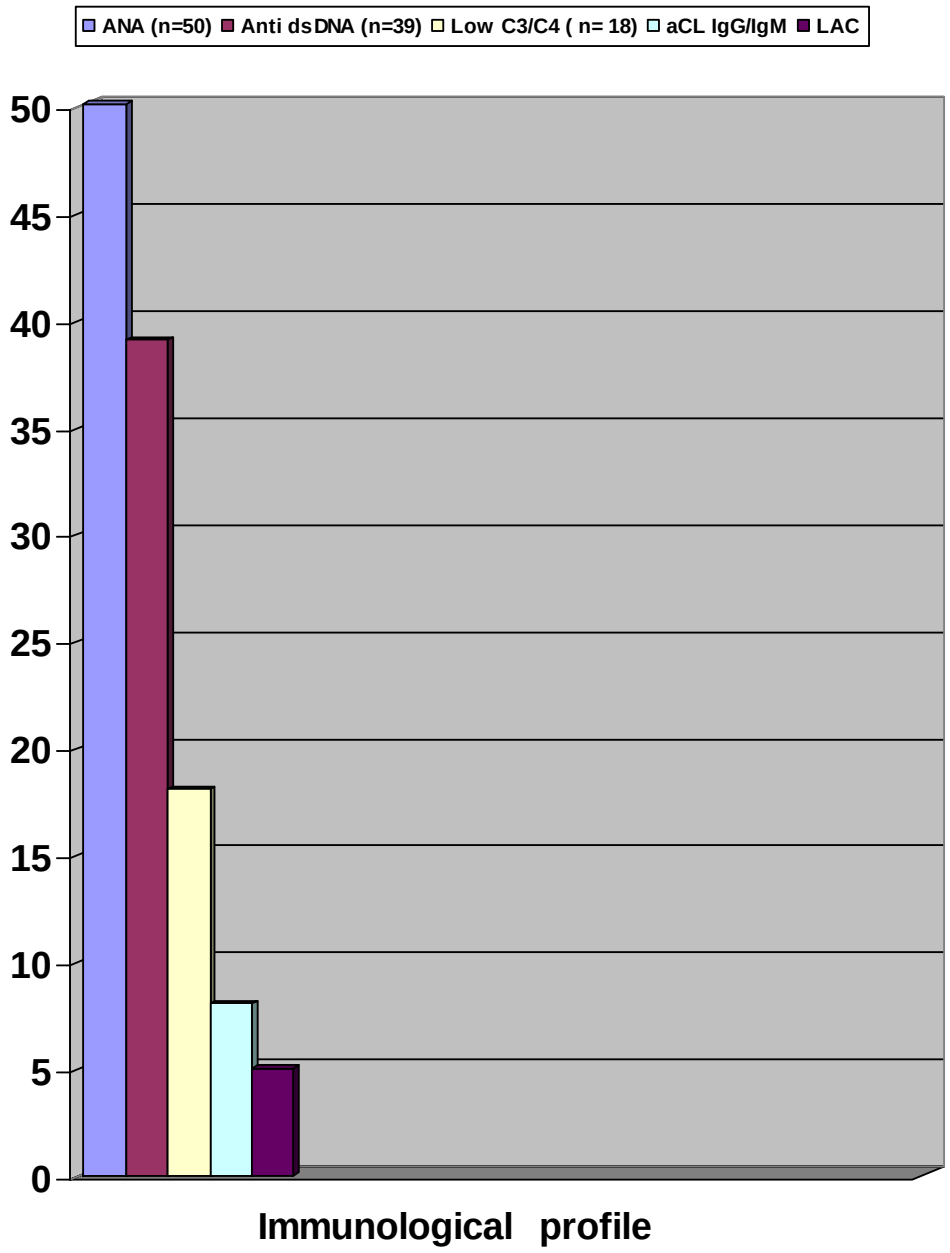
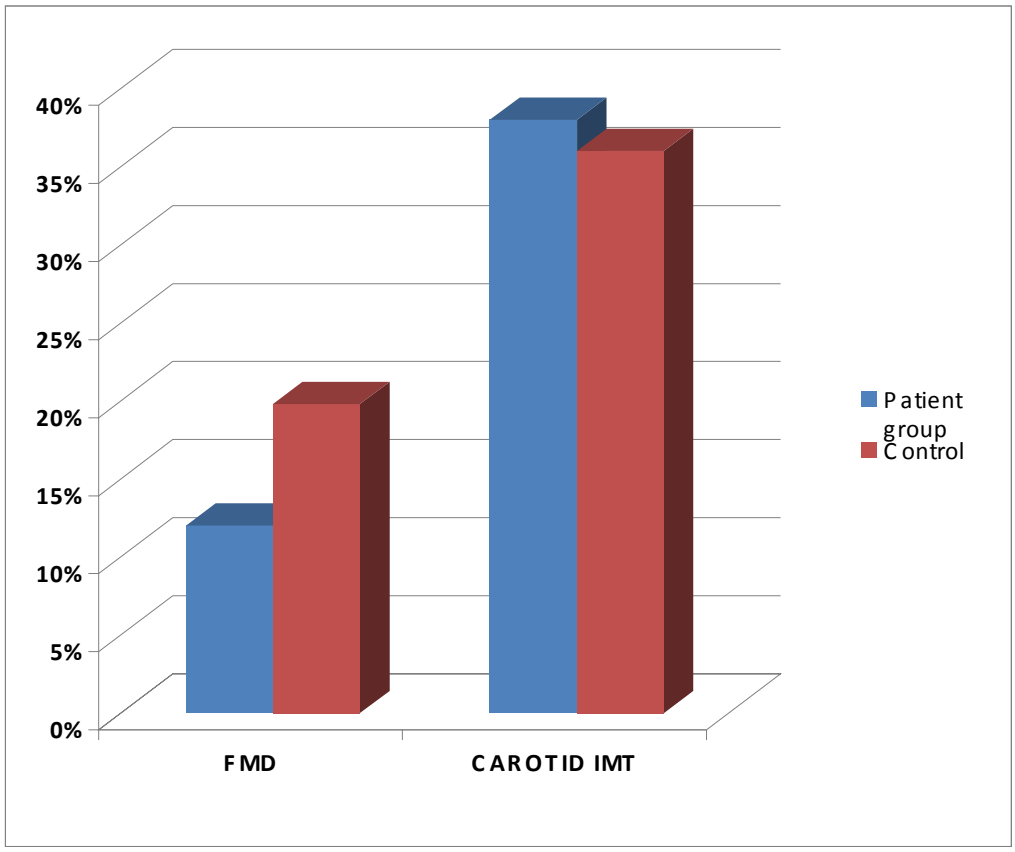

ANA (n=50)
Anti dsDNA (n=39)
Low C3/C4 (n= 18)
aCL IgG/IgM
LAC
50
45
40
35
30
25
20
15
10
5
0
Immunological profile

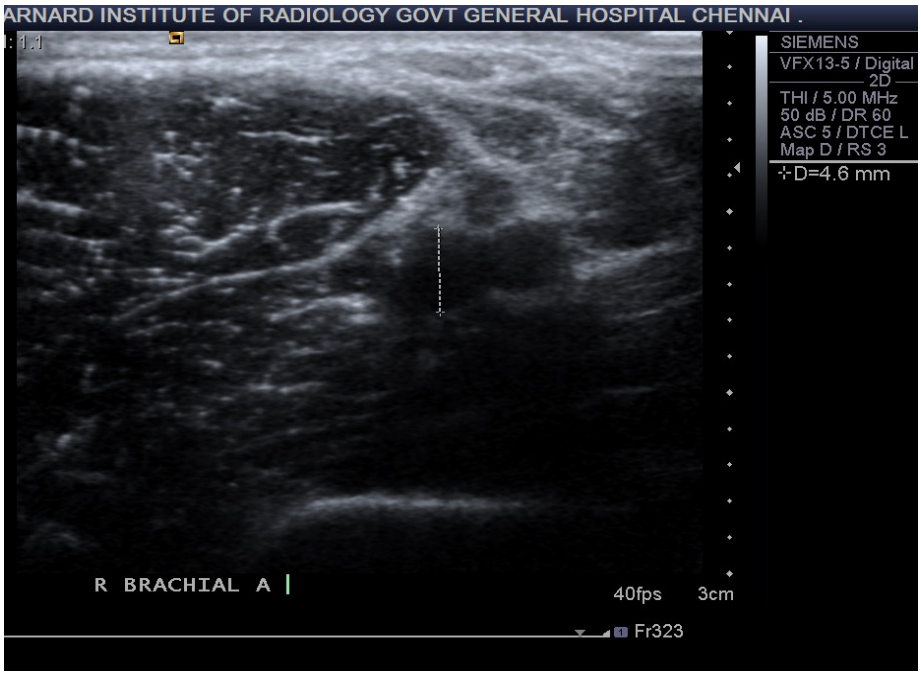
TABLE 3: Study characteristics in the patient and control group

Parameter	Patient group	Control group	p value
Baseline Diameter (mm)	3.64± 0.40	3.67 ± 0.34	0.64
FMD (%)	12 ± 3.92	19.8 ± 2.27	< 0.001
Carotid IMT (mm)	0.38	0.36	0.09

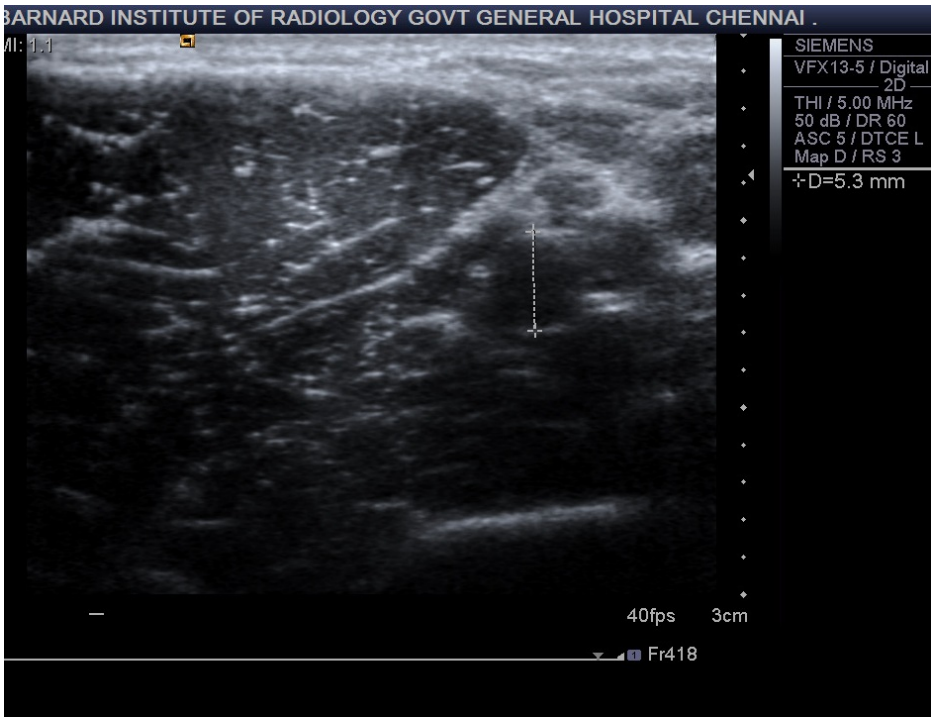
The patients and the controls had a similar degree of baseline diameter of the brachial artery (3.64 ± 0.40 vs. 3.67 ± 0.34 mm, p = 0.64) and carotid IMT (0.38 ± 0.08 vs. 0.36 ± 0.06, p value 0.09). However, the SLE patients had worse endothelial function than the controls (FMD 12 ± 3.92% vs. 19.8 ± 2.27%, p<0.001). The endothelial function, assessed by vascular ultrasonography on brachial artery, is shown (Baseline **Photo (C)** & post dilatation **Photo (D).** The carotid IMT measurement is shown in **Photo (E).**


40%
35%
30%
25%
20%
15%
10%
5%
0%
FMD
CAROTID IMT
Patient group
Control

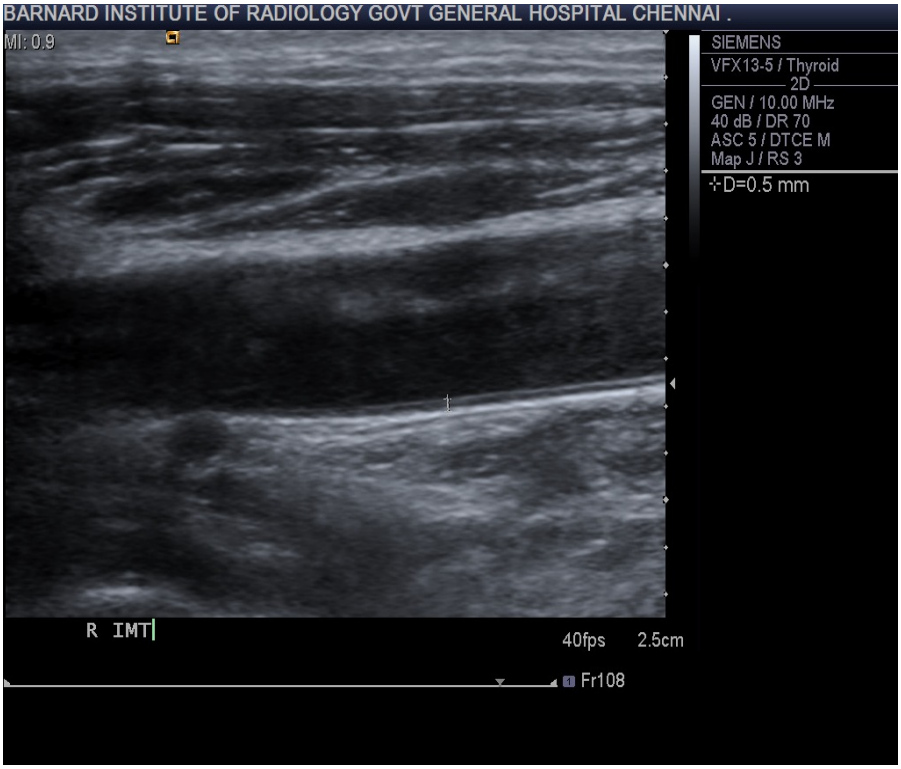
C)


ARNARD INSTITUTE OF RADIOLOGY GOVT GENERAL HOSPITAL CHENNAI .
SIEMENS
VFX13-5 / Digital
2D
THI / 5.00 MHz
50 dB / DR 60
ASC 5 / DTCE L
Map D / RS 3
D=4.6 mm
R BRACHIAL A
40fps
3cm
Fr323

(D)


ARNARD INSTITUTE OF RADIOLOGY GOVT GENERAL HOSPITAL CHENNAI .
SIEMENS
VFX13-5 / Digital
2D
THI / 5.00 MHz
50 dB / DR 60
ASC 5 / DTCE L
Map D / RS 3
D=5.3 mm
40fps
3cm
Fr418

(E)


BARNARD INSTITUTE OF RADIOLOGY GOVT GENERAL HOSPITAL CHENNAI .
MI: 0.9
SIEMENS
VFX13-5 / Thyroid
2D
GEN / 10.00 MHz
40 dB / DR 70
ASC 5 / DTCE M
Map J / RS 3
D=0.5 mm
R IMT
40fps
2.5cm
Fr108

Correlations between FMD and SLE parameters:

The correlations between FMD and biological and immunological parameters are shown in Table 4.

TABLE 4: Parameter Correlation coefficient

Disease duration	**r = 0.0998**	**p = 0.49**
Carotid IMT	**r = – 0.1993**	**p = 0.16**
SLEDAI	**r = – 0.5678**	**p = <0.01**
C3	**r = 0.9101**	**p = < 0.01**
Anti dsDNA antibodies	**r = – 0.8658**	**p = <0.01**
Total cholesterol	**r = 0.0239**	**p = 0.86**
ACL IgG	**r = 0.2388**	**p = 0.09**
ACL IgM	**r = 0.2149**	**p = 0.13**

The statistical analysis showed an inverse correlation between FMD and antidsDNA and SLEDAI which is significant statistically. Similarly there is strong positive correlation between FMD and C3. But, correlation does not imply causation.

The size of any correlation generally evaluates as follows:

Value of r	Qualitative Description of the Strength
–1	perfect negative
(–1, –0.75)	strong negative
(–0.75, –0.5)	moderate negative
(–0.5, –0.25)	weak negative
(–0.25, 0.25)	no linear association
(0.25, 0.5)	weak positive
(0.5, 0.75)	moderate positive
(0.75, 1)	strong positive
1	perfect positive

DISCUSSION

DISCUSSION

The patients with SLE have a high incidence of atherosclerosis with its main consequence: coronary artery disease. Epidemiological studies have shown that SLE women aged 35 – 44 years were over 50 times more likely to develop myocardial infarction than women of similar age from general population **(3)**. Anatomo-pathological investigations have revealed that the SLE patients were prone to develop a premature atherosclerosis **(102)**. The increased risk of atherosclerosis is not exclusively related to traditional risk factors alone **(103)**. In the last years, SLE itself appeared like an independent risk factor for atherosclerosis, acting through autoimmune vascular injury **(6)**. In patients with systemic lupus erythematosus, atherosclerosis has a long period of subclinical evolution. The first reversible step in the atherogenesis process is represented by the endothelial dysfunction **(104)**.

Endothelial dysfunction is well described entity in connective tissue diseases, leading to accelerated atherosclerosis and associated morbidity and early mortality. There are several associated factors like body mass index, co morbidities, immunosuppressants, dyslipidemia, hypertension which could contribute to the endothelial dysfunction. Our study was designed to first identify the prevalence of this in our patients and more importantly attempt to delineate the disease contribution from all associated factors. In this regard, we have chosen newly diagnosed cases who have not been on treatment and have excluded other associated factors like hypertension, dyslipidemia & diabetes mellitus. We could thereby to an extent, isolate the disease related factor and attribute the endothelial dysfunction to it.

Endothelial dysfunction appears when the normal functions of the endothelial cells (control of vascular tone and blood pressure, regulation of leucocytes traffic from the blood to the tissues, and platelet adhesion and aggregation, maintenance of the balance between blood coagulation and fibrinolysis, control of growth, development and differentiation of the vessel wall cells) are lost or dysregulated **(105)**. A non-invasive method for the assessment of endothelial dysfunction is represented by flow mediated vasodilation (endothelium dependent dilation) **(106)**. In our study, FMD in SLE patients was significantly lower than in control subjects (FMD 12±3.92% vs. 19.8±2.27%, p<0.001). There are several studies with similar results, starting with the first study, performed by **Lima *et al*. (107)**, who showed that SLE patients presented lower FMD than sex and age-matched controls (5.0±5.0% vs. 12.0± 6.0%) , even in subjects without traditional cardiovascular risk factors **(70)**. **Tani *et al*. (16)** identified the same pattern of FMD in SLE patients as well as by **Valdivielso et al (68)** (FMD 12.4+/-4.4% vs. 16.9+/-5.5%, p<0.05). The reduced values of FMD in patients with SLE were found by **Piper and Turner** in their studies, too **(67, 71)**. Our study concurred with the FMD results of the other indian study done by **Parasar Ghosh et al (73)** which was impaired in SLE patients compared to controls (9.97% vs. 18.97%, $p<0.00001$).

The Carotid IMT values showed no significant difference in our study and control population (0.38 ± 0.08 vs. 0.36 ± 0.06, p value 0.09). Similar results were obtained in the study done by **Valdivielso et al (68)** (0.58+/-0.08 mm vs. 0.57+/-0.07 mm, NS). **Parasar Ghosh et al (73)** have reported higher carotid IMT in SLE patients as compared to controls (0.49±0.08 mm vs. 0.39±0.05 mm, $p<0.0001$) and

had shown a good negative correlation between brachial FMD and carotid IMT, which was not the case in our study. **Masoud El-Magadmi et al (67) & Zahra Seyyedbonakdar et al (69)** also observed a negative correlation with percent FMD in their studies. The reason for this difference could be that in early stages of the disease to which our patients belonged to, identification of a functional abnormality (FMD) would have preceded the structural changes that occur in the carotid arteries in the form of intima medial thickness that occurs on a later stage. Moreover the other factors which would contribute to subclinical atherosclerosis like comorbities, immunosuppressants were not excluded in their studies as against ours.

We found a moderate negative correlation between FMD and SLEDAI ($r = -0.7321$, $p < 0.001$). This correlation between FMD and the disease activity was identified in other studies **(96, 108 & 109)**. **Valdivielso et al (68)** also have reported significant correlation between FMD and LAI (Lupus Activity Index). AntidsDNA had a strong negative correlation, while C3 complement assay had strong positive correlation with flow mediated dilatation. There was no linear correlation between other factors like disease duration, cholesterol, anticardiolipin antibodies.

When comparing our study with the other indian study done by **Parasar Ghosh et al (73)** regarding other factors, our study population has less disease duration (mean 7.3 months) when compared to their patient population (mean 60 months). Similarly their patient population had co morbidities (hypertension, diabetes mellitus, dyslipidemia), and had been exposed to several immunosuppressants. These factors have their contribution in the endothelial dysfunction.

FMD using vascular ultrasonography on brachial artery represents a useful, non-invasive method for the assessment of the endothelial dysfunction. Reactive hyperemia produces a shear stress stimulus that induces the normal endothelium to release nitric oxide (NO), which acts as a vasodilator. Impaired endothelial function is associated with a reduced release of NO and a lower vasodilation **(110)**. The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is under explored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature.

Ongoing studies in several large populations, including the Framingham Heart Study and the Cardiovascular Health Study, shall determine whether endothelial dysfunction in the brachial artery will identify patients at risk for developing coronary artery disease, cerebral vascular disease and/or peripheral vascular disease. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention.

Numerous studies have demonstrated that brachial artery reactivity improves with risk factor modification and treatment with drugs known to reduce cardiovascular risk. It remains unknown whether an improvement in endothelial function directly translates into improved outcome. In the future, however, practitioners may use brachial artery FMD to assess response to drug therapy and to individualize patient risk factor modification programs. Further studies are needed to determine whether the methodology is sufficiently reproducible and whether

biological variability is sufficiently low to make assessment of FMD a clinically useful measure of cardiovascular risk on an individual or group basis. To that end, the methodology will need to mature, with formal opportunities for training, certification and continuing medical education, as currently exist for other cardiovascular testing modalities.

FUTURE DIRECTIONS

Ultrasound assessment of brachial artery FMD has yielded important information about vascular function in health and disease, yet several new approaches and technological advances have emerged. Most prior studies examined FMD at a single time point, typically 1 min after cuff release. This practice evolved from the observations that the maximal dilator response occurs at approximately 1 min in healthy subjects **(111)** and that the necessity for manual acquisition and measurement placed a practical limit on the number of image frames that could be analyzed. Commercially available technology now makes it possible to acquire multiple images of the brachial artery automatically using the ECG signal as a trigger and to measure arterial diameter automatically using computer-based edge detection techniques. This approach allows investigators to examine the entire time course of brachial dilation in response to reactive hyperemia , the true peak response, the time to peak and the overall duration of FMD as discussed in the previous text. The time course and extent of brachial expansion within a single cardiac cycle, possibly reflecting vessel compliance, can be examined. In the carotid artery, compliance has been shown to correlate with cardio- vascular risk **(112)**. About 70% of the dilation observed 1 min after cuff release is attributable to NO synthesis.

Further studies are needed to evaluate other vasoactive mechanisms and to determine whether various disease states influence the kinetics and/or extent of FMD. Careful examination of the vasodilator response to NTG provides another potential avenue for investigation. Although most studies have detected little effect

of disease states on this response, there is evidence that cardiovascular risk factors might impair the vasodilator response to NTG, especially when a dose-response curve is measured. These findings are consistent with experimental studies demonstrating that inactivation of NO by reactive oxygen species is an important mechanism of vascular dysfunction. Further information about the causes of vascular dysfunction and the response to interventions may be gained by examining the response to a sub maximal dose of NTG or a series of NTG doses. The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is under explored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature.

CONCLUSION

CONCLUSION

1) Endothelial dysfunction is present in SLE patients even in the absence of traditional cardiovascular risk factors.

2) FMD using vascular ultrasonography on brachial artery represents a non-invasive, reproducible and cost effective tool for the assessment of endothelial dysfunction.

3) Impaired flow mediated dilatation of brachial artery may be an early physiological feature of endothelial dysfunction and may precede the anatomical increase in carotid intimal thickness.

4) FMD of brachial artery has significant negative correlation with disease activity and antidsDNA antibody.

5) Future prospects of FMD technique include

   a) early screening module for endothelial dysfunction and to start on drugs with pleotrophic effect like statins, ACE inhibitors and Aspirin.

   b) serial screening method to ascertain improvement while on treatment with immunosuppressive agents and vasodilators.

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APPENDICES

ABBREVIATIONS

SLE	Systemic lupus erythematosus
FMD	Flow-mediated vasodilatation
CVD	Cardiovascular disease
IMT	Intima-media thickness
LAI	Lupus activity index
EDD	Endothelial dependent vasodilatation
BMI	Body mass index
SLEDAI	SLE Disease Activity Index
SLICC	Systemic Lupus International Collaborating Clinics
ESR	Erythrocyte sedimentation rate
CRP	C Reactive protein
ANA	Anti nuclear antibody
Anti dsDNA	Anti double stranded DNA
aCL	Anticardiolipin antibody

PROFORMA

NAME: **AGE/SEX:** **OP/ IP No:**

RCC No :

ADDRESS: **OCCUPATION:**

H/O PRESENT ILLNESS: **TOTAL DURATION OF ILLNESS**

Fever	Malaise	Fatigue
Malar rash	Discoid lesion	Oral ulcer
Alopecia	Photosensitivity	
Purpura	Raynaud's	Gangrene
Joint Symptoms		
Myalgia	Weakness	Headache
Visual sym	Mood	Seizures
Chest pain	Palpitation	Dyspnoea
Syncope	Pedal edema	
Cough	Expectoration	Hemoptysis
Hematuria	Oliguria	Facial puffiness

OTHERS

PAST HISTORY:

PERSONAL HISTORY:

TREATMENT HISTORY:

PHYSICAL EXAMINATION:

Fever Anaemia clubbing cyanosis LN PE JVP

MUCOCUTANEOUS

OTHERS

PULSE BP RR
CVS RS ABDOMEN

CNS Fundus: MSS:

INVESTIGATIONS:

Hb TC DC ESR 1 hr Platelets
BT CT PT INR APTT
Urea Cr Uric acid Sugar T.Bilirubin
ALT AST
SAP LDH CPK Na K HCO 3 Cl
Lipid profile
Urine R/E PCR 24 hrs protein
ANA Anti dsDNA ACL
LAC VDRL CRP
Complement ENA

Renal Biopsy :

ECG CXR PA View
ECHO Carotid IMT

Flow Mediated Dilatation **Baseline** **FMD**

ASSESSMENT SLEDAI SLICC

MANAGEMENT

NSAIDS STEROIDS Pulse
Oral
IMMUNOSUPPRESSANTS

ANTICOAGULANTS/ANTIPLATELETS

SLEDAI

Systemic Lupus Erythematosus Disease Activity Index

Descriptor	Definition	Weighted Score
Seizure	Recent onset; exclude metabolic, infectious, or drug-related causes	8
Psychosis	Altered ability to function in normal activity owing to severe disturbance in the perception of reality; includes hallucinations, incoherence marked by loose associations, impoverished thought content, marked illogical thinking, and bizarre disorganized or catatonic behavior; exclude the presence of uremia and offending drugs	8
Organic brain syndrome	Altered mental function with impaired orientation or impaired memory or other intellectual function, with rapid onset and fluctuating clinical features; includes clouding of consciousness with reduced capacity to focus and inability to sustain attention on environment, and at least two of the following—perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, and increased or decreased psychomotor activity; exclude metabolic infectious and drug-related causes	8
Visual	Retinal changes from systemic lupus erythematosus cytoid bodies, retinal hemorrhages, serous exudate or hemorrhage in choroid, optic neuritis (not due to hypertension, drugs, or infection)	8
Cranial nerve	New onset of sensory or motor neuropathy involving a cranial nerve	8
Lupus headache	Severe, persistent headache; may be migrainous, unresponsive to narcotic analgesia	8
Cerebrovascular accident	New syndrome; exclude arteriosclerosis	8
Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages; vasculitis confirmed by biopsy or angiogram	8
Arthritis	More than two joints with pain and signs of inflammation (tenderness, swelling, or effusions)	4

Descriptor	Definition	Weighted Score
Myositis	Proximal muscle aching or weakness associated with elevated creatine phosphokinase/aldolase levels, electromyographic changes, or biopsy specimen showing myositis	4
Casts	Heme, granular, or erythrocyte	4
Hematuria	>5 erythrocytes per high-power field; exclude other causes (stone, infection)	4
Proteinuria	>0.5 g of urinary protein excreted per 24 hr; new onset or recent increase of >0.5 g/24 hr	4
Pyuria	>5 leukocytes per high-power field; exclude infection	4
New malar rash	New onset or recurrence of inflammatory type of rash	4
Alopecia	New or recurrent; patch of abnormal, diffuse hair loss	4
Mucous membrane	New onset or recurrence of oral or nasal ulceration	4
Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening	4
Pericarditis	Pericardial pain with at least one rub or effusion; confirmation by ECG or echocardiography	4
Low complement	Decrease in CH50, C3, or C4 levels (to less than the lower limit of the laboratory-determined normal range)	2
Increased DNA binding	>25% binding by Farr assay (to more than the upper limit of the laboratory-determined normal range, e.g., 25%)	2
Fever	>38°C after exclusion of infection	1
Thrombocytopenia	<100,000 platelets	1
Leukopenia	Leukocyte count <3000/mm^3 (not due to drugs)	1

SLICC
Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for Systemic Lupus Erythematosus

Item	Score
Ocular (either eye by clinical assessment)	
Any cataract ever	0, 1
Retinal change or optic atrophy	0, 1
Neuropsychiatric	
Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) or major psychosis	0, 1
Seizures requiring therapy for 6 mo	0, 1
Cerebrovascular accident ever (score 2 if >1)	0, 1, 2
Cranial or peripheral neuropathy (excluding optic)	0, 1
Transverse myelitis	0, 1
Renal	
Estimated or measured glomerular filtration rate <50%	0, 1
Proteinuria >3.5 g/24 hr	0, 1
or End-stage renal disease (regardless of dialysis or transplantation)	*or* 3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P_2)	0, 1
Pulmonary fibrosis (physical and radiographic)	0, 1
Shrinking lung (radiograph)	0, 1
Pleural fibrosis (radiograph)	0, 1
Pulmonary infarction (radiograph)	0, 1
Cardiovascular	
Angina or coronary artery bypass	0, 1
Myocardial infarction ever (score 2 if >1)	0, 1, 2
Cardiomyopathy (ventricular dysfunction)	0, 1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	0, 1
Pericarditis for 6 mo or pericardiectomy	0, 1
Peripheral vascular	
Claudication for 6 mo	0, 1
Minor tissue loss (pulp space)	0, 1

Significant tissue loss ever (e.g., loss of digit or limb) (score 2 if >1 site)	0, 1, 2
Venous thrombosis with swelling, ulceration, or venous stasis	0, 1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver or gallbladder ever, for any cause (score 2 if >1 site)	0, 1, 2
Mesenteric insufficiency	0, 1
Chronic peritonitis	0, 1
Stricture or upper gastrointestinal tract surgery ever	0, 1
Chronic pancreatitis	0, 1
Musculoskeletal	
Muscle atrophy or weakness	0, 1
Deforming or erosive arthritis (including reversible deformities, excluding avascular necrosis)	0, 1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	0, 1
Avascular necrosis (score 2 if >1)	0, 1, 2
Osteomyelitis	0, 1
Tendon rupture	0, 1
Skin	
Scarring chronic alopecia	0, 1
Extensive scarring of panniculus other than sculp and pulp space	0, 1
Skin ulceration (excluding thrombosis for >6 mo)	0, 1
Premature gonadal failure	0, 1
Diabetes (regardless of treatment)	0, 1
Malignancy (exclude dysplasia) (score 2 if >1 site)	

CONSENT FORM

PATIENT CONSENT FORM

STUDY TITLE
ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSIS

Study Centre : Department of Rheumatology,
Madras Medical College, Chennai – 600 003

Patient's Name :
Patient's Age :
Identification Number : Patient may check (✓) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethics committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and to faithfully co-operate with the study team, and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ☐

I hereby consent to participate in this study on **ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSIS** ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological and urine examination. ☐

Signature / Thumb Impression _____ Place _____ Date _____

Patient's Name and Address: _____

Signature of the Investigator : _____ Place _____ Date _____

Study Investigator's Name : _____

ETHICAL COMMITTEE APPROVAL

INSTITUTIONAL ETHICAL COMMITTEE
GOVERNMENT GENERAL HOSPITAL & MADRAS MEDICAL COLLEGE,
CHENNAI-600 003.

Telephone: 044-2530 5000
Fax : 044 – 25305113

K.Dis.No. 0029094/P & D3/Ethies/Dean/GGH/09 Dated: 10/2/2009

Title of the work : "Endothelial dysfunction in systemic Lupus Erythematosis"

Principal Investigator : Dr. S. Rajesh M.D.,

Department : Rheumatology, MMC, ch-3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on . 17 – 2 – 2009 at 2 P.M in Government General Hospital, Deans, Chamber, Chennai-3.

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The principal investigator and their term are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate form the area of the work for which I applied for ethical clearance
5. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
6. You should abide to the rules and regulations of the institution(s)
7. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

SECRETARY
IEC, GGH,CHENNAI

CHAIRMAN
IEC, GGH,CHENNAI

DEAN
GGH &MMC, CHENNAI

Rkm.5.9(2)

S.No	RCC	Age	Sex	Duration (mths)	FMD %	IMT mm	chol mg	TGL	HDL	LDL	anti dsDNA
1	52067	20	F	12	12.2	0.4	187	70	42	80	1.28
2	49464	28	F	6	12.5	0.5	180	130	42	106	1.48
3	50636	17	F	6	8.3	0.4	167	111	40	88	4.43
4	51204	17	F	12	12.1	0.4	146	88	46	90	1.38
5	51109	30	F	12	10.5	0.3	166	112	46	96	2.42
6	49417	16	F	8	17.8	0.3	171	130	40	102	0.49
7	51244	35	F	10	11.4	0.4	160	106	40	113	1.64
8	51270	32	F	8	14.7	0.5	185	110	42	120	0.54
9	49585	19	F	12	16.1	0.3	196	110	40	120	0.57
10	51105	17	F	5	24.2	0.3	197	123	51	113	0.37
11	51238	23	F	3	8.6	0.3	196	108	42	102	2.5
12	51242	22	F	7	18.1	0.3	166	128	43	113	0.46
13	51223	24	M	4	15.2	0.4	161	124	43	128	0.63
14	51188	17	M	8	12.1	0.4	176	130	42	110	1.38
15	51304	28	F	12	12.2	0.5	140	115	40	115	0.36
16	49905	27	F	12	17.1	0.4	180	124	50	110	1.48
17	51242	35	F	6	18.4	0.4	146	127	41	102	1.59
18	51336	25	F	14	16.2	0.3	137	108	42	118	0.56
19	51305	29	F	12	9.8	0.4	191	113	49	120	2.78
20	51246	23	F	3	13.5	0.3	138	107	42	90	0.3
21	50785	23	F	14	16.2	0.3	110	105	41	120	0.54
22	49589	31	F	5	12.8	0.4	174	126	45	102	1.51
23	51214	27	F	2	12.2	0.4	187	127	56	112	1.42
24	51200	25	F	12	12.5	0.3	170	122	43	102	1.54
25	51360	34	F	6	7.5	0.3	146	100	46	102	3.37
26	50955	17	F	6	16.2	0.4	146	110	40	122	0.42

S.No	RCC	Age	Sex	Duration (mths)	FMD %	IMT mm	chol mg	TGL	HDL	LDL	anti dsDNA
27	51703	20	F	3	16.1	0.5	189	133	40	114	0.49
28	51125	21	F	6	7.5	0.6	192	112	42	112	3.98
29	50344	20	F	10	4.9	0.5	124	108	42	86	5.14
30	51388	23	F	5	4.9	0.4	194	130	46	112	4.94
31	51449	21	F	12	7.5	0.4	140	110	42	96	3.98
32	51399	17	F	4	7.9	0.3	146	110	47	112	3.45
33	51445	20	F	6	7.5	0.3	192	106	44	122	3.06
34	51472	19	F	5	6.5	0.3	132	124	52	112	4.2
35	51531	18	F	4	7.9	0.4	198	122	42	110	3.89
36	51648	21	F	12	12.1	0.3	188	113	47	102	1.59
37	51622	21	F	6	13.3	0.4	182	113	41	102	1.38
38	50098	18	F	12	10.8	0.4	194	93	47	113	2.56
39	49510	16	F	6	8.8	0.4	192	120	42	102	3.05
40	51762	25	F	12	8.6	0.4	172	113	42	102	3.26
41	51851	27	F	3	11.8	0.3	177	124	44	114	2.32
42	51831	27	F	6	14.2	0.4	169	114	46	110	0.8
43	51891	28	F	1	8.6	0.6	170	113	42	102	3.42
44	51911	18	F	12	13.3	0.3	195	125	40	122	1.28
45	51630	25	F	3	12.9	0.4	118	108	46	108	1.18
46	51977	20	F	3	12.9	0.4	188	108	45	112	1.3
47	51278	29	F	3	15.2	0.5	188	102	42	106	0.8
48	51983	19	F	4	12.1	0.3	148	114	45	106	1.47
49	51779	23	F	3	7.3	0.5	180	122	41	122	5.14
50	52067	20	F	6	10	0.3	151	102	41	88	2.28

S. No	RCC NO	ACLIgG	ACLIgM	C3 (mg/dl)	SLEDAI	SLICC	Renal
1	52067	6	9	-	3	0	class 2/5
2	49464	19	30.9	-	12	1	N
3	50636	8	6	57.7	17	0	N
4	51204	15.8	20	-	13	0	N
5	51109	4.1	9.4	-	8	0	N
6	49417	2.9	5.4	-	5	0	N
7	51244	9.4	4.8	82	13	0	N
8	51270	4.6	3.9	-	16	0	N
9	49585	16.4	15.6	-	8	1	N
10	51105	15.6	19	-	13	0	N
11	51238	8	1.7	64	29	0	N
12	51242	23.7	20	144	7	0	N
13	51223	3.2	2.9	126	9	0	N
14	51188	3.7	4.1	-	11	0	N
15	51304	5.8	4.6	-	11	0	N
16	49905	3.5	7.1	166	2	0	N
17	51242	3.2	2.2	-	7	0	N
18	51336	8	6.3	-	12	0	N
19	51305	20.7	35	78	16	0	N
20	51246	3	7.1	-	10	0	N
21	50785	5.8	2.9	-	13	0	N
22	49589	11	7.1	166	6	0	N
23	51214	6	4.6	-	11	0	N

S. No	RCC NO	ACLIgG	ACLIgM	C3 (mg/dl)	SLEDAI	SLICC	Renal
24	51200	4.1	5.1	-	10	0	N
25	51360	2.1	9	48	14	0	N
26	50955	3.5	10	138	13	0	N
27	51703	6.1	5.1	-	3	0	N
28	51125	4	6.3	-	16	0	N
29	50344	5.7	4.6	42	26	0	N
30	51388	8	2.2	38	24	0	N
31	51449	4.6	5.3	64	10	0	N
32	51399	3.2	2.7	-	17	0	N
33	51445	5.7	5.3	-	11	0	N
34	51472	4.2	6.3	-	14	0	N
35	51531	6	4.4	52	16	0	N
36	51648	8	4.8	-	4	0	N
37	51622	23.7	15.6	166	5	0	N
38	50098	3.2	6	-	15	1	N
39	49510	5	2.2	-	17	0	N
40	51762	4.1	9	78	9	0	N
41	51851	8	11.2	-	12	0	N
42	51831	33.1	15.6	168	11	0	N
43	51891	4.7	5.4	72	12	0	N
44	51911	5.8	6.6	-	9	0	N
45	51630	3.2	1.7	-	9	0	N
46	51977	8	7.1	152	5	0	N

S. No	RCC NO	ACLIgG	ACLIgM	C3 (mg/dl)	SLEDAI	SLICC	Renal
47	51278	4.7	4	-	5	0	N
48	51983	3.7	4.8	-	8	0	N
49	51779	5.8	2.7	58	16	0	N
50	52067	6.9	12	87	17	0	N

S. No.	OP	AGE	SEX	FMD (%)	IMT (mm)	CHOL	TGL	HDL	LDL
1	15666	23	F	14.3	0.5	183	124	47	110
2	15668	31	F	17.1	0.4	176	126	44	120
3	15670	27	F	22.9	0.4	183	123	46	102
4	15678	25	F	20	0.5	186	128	43	106
5	15789	34	F	18.9	0.4	184	120	40	110
6	15899	17	F	21.4	0.3	190	110	41	102
7	15896	20	F	19.5	0.4	176	110	46	106
8	15898	21	F	22.8	0.5	194	116	46	110
9	15977	20	F	17.2	0.3	174	122	47	106
10	16003	23	F	21.8	0.3	176	130	47	104
11	16024	21	F	21.6	0.4	183	112	42	110
12	16026	17	F	22.6	0.3	170	116	46	102
13	16027	20	F	18.6	0.4	186	124	41	110
14	16033	19	F	20	0.3	183	126	47	108
15	16035	18	F	22.9	0.3	156	106	42	102
16	16045	21	F	20	0.4	170	126	46	112
17	16098	21	F	21.6	0.4	186	122	42	110
18	16094	18	F	21	0.4	196	120	44	110
19	16108	16	F	21.2	0.4	186	102	46	106
20	16118	25	F	22.2	0.3	176	108	42	106
21	16128	27	F	20.2	0.4	196	126	42	110
22	16146	27	F	20	0.4	176	110	44	102
23	16154	28	F	22.5	0.4	184	126	40	102
24	16124	18	F	17.5	0.3	186	110	44	112
25	16134	25	F	17.9	0.4	146	126	40	102

S. No.	OP	AGE	SEX	FMD (%)	IMT (mm)	CHOL	TGL	HDL	LDL
26	16213	20	F	17.5	0.3	146	114	42	110
27	16154	29	F	22	0.3	189	110	40	102
28	16166	19	F	17.1	0.3	192	116	46	106
29	16178	23	F	21.2	0.4	124	123	40	112
30	16188	17	F	21.4	0.3	194	122	42	110
31	16192	20	F	21.2	0.4	140	102	42	84
32	16198	28	F	22.2	0.3	146	96	40	110
33	16233	17	F	18.8	0.4	192	112	40	122
34	16234	14	F	22.9	0.3	132	114	42	106
35	16355	30	F	20	0.3	198	106	44	86
36	16557	16	M	17.6	0.3	188	104	40	106
37	16559	36	F	18.4	0.3	182	108	40	112
38	16568	32	F	18.4	0.4	194	106	46	120
39	16572	19	F	19.4	0.3	192	102	42	112
40	16576	17	F	15.8	0.3	172	98	42	90
41	16582	23	F	14.6	0.4	177	102	40	106
42	16590	22	F	17.9	0.3	169	84	42	102
43	16592	24	M	15.2	0.3	170	110	48	110
44	16595	17	M	22.6	0.4	195	102	40	102
45	16598	28	F	21.9	0.4	118	106	44	90
46	16599	27	F	20	0.3	188	110	40	98
47	16702	35	F	19.4	0.4	188	108	42	113
48	16744	25	F	19.4	0.3	148	106	43	90
49	16746	29	F	20	0.4	180	113	41	86
50	16748	23	F	21.2	0.4	151	96	40	88

ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

D.M BRANCH IX -RHEUMATOLOGY

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY
CHENNAI – 600032

AUGUST 2010

CERTIFICATE

This is to certify that the dissertation entitled "**ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS"** presented here is the original work done by **Dr.S.Rajesh**, postgraduate in the Department of Rheumatology, Madras Medical College & Government General Hospital, Chennai-600003, in partial fulfillment of the University rules and regulations for the award of **D.M BRANCH IX –RHEUMATOLOGY,** under my guidance and supervision during the academic period from 2007- 2010.

Dr. J. Mohanasundaram MD, Ph D, D N B.,
Dean
Madras Medical College &
Government General Hospital,
Chennai -3

Dr. R.Porkodi,MD,DM.,
Professor & Head,
Dept of Rheumatology,
Madras Medical College &
Govt General Hospital, Ch-3

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CONTENTS

INTRODUCTION

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease, with a wide range of clinical manifestations. In 1976, **Urowitz *et al.* (1)** postulated a bimodal mortality pattern in patients with this disease: in the first part of evolution, mortality is due to severe infections or to disease activity, but after 5 years of SLE course, mortality is caused by the accelerated atherosclerosis and its consequences. With the constantly increasing drugs available in the therapeutic armamentarium, even though the early mortality has been brought under control, the late mortality and morbidity associated with SLE remains at high levels. During the last 3 decades, there have been several studies on atherosclerosis in SLE. It has been proved that atherosclerosis has a high incidence among young women with SLE. These patients have a high prevalence of coronary artery disease and an incidence of myocardial infarction up to 50 times higher than age-matched healthy subjects. This high incidence of atherosclerosis in SLE cannot be attributed only to traditional risk factors **(2, 3)**.

Endothelial function is thought to be an important factor in the pathogenesis of atherosclerosis, hypertension and heart failure. In healthy subjects, endothelium is more than a physical barrier and has several functions, like: a) continuous regulation of vascular tone, b) leucocytes adhesion, c) maintenance of the balance between thrombotic and anticoagulant properties of the blood **(4)**. When these functions of the endothelium are affected, endothelial dysfunction appears.

Endothelial dysfunction is considered as the first step in the atherogenetic process. Endothelial dysfunction in SLE is produced by the clustering of traditional risk factors, adverse effects of treatment and SLE itself as an independent risk factor **(5, 6)**. Systemic inflammation, autoantibodies directed to double stranded DNA (dsDNA), ribonucleoproteins (nRNP), endothelial cells, phospholipids, circulating immune complexes, activated complement products, lupus nephropathy and dyslipidemia represent some factors related to SLE which contribute to appearance of endothelial dysfunction **(7,8)**. In the 1990s, high-frequency ultrasonographic imaging of the brachial artery to assess endothelium-dependent flow-mediated vasodilatation (FMD) was developed. The technique provokes the release of nitric oxide, resulting in vasodilatation that can be quantitated as an index of vasomotor function. The noninvasive nature of the technique allows repeated measurements over time to study the effectiveness of various interventions that may affect vascular health **(9)**.

Tremendous interest exists in determining the clinical utility of brachial artery FMD. Investigators have hypothesized that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a preclinical marker of cardiovascular disease **(10)**. Several studies suggest that the presence of endothelial dysfunction in the coronary circulation is an independent predictor of cardiovascular disease events **(11, 12)**. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention. Recently, endothelial dysfunction has also been found in patients with systemic vasculitis and has been reversed by administration of immunosuppressive

therapy **(13)**. As endothelial dysfunction may represent an early stage in atherogenesis, it is important to understand the mechanisms of its development in a condition such as SLE. It is also important to determine whether it is associated with other CHD risk factors or early atheroma.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

SLE predominantly occurs in women, with a gender ratio of 9:1. Onset is typically in the age group of 20- 30 yrs. Multiple predisposing factors have been identified. The genetic predisposition is complex, likely involving more than 100 genes. HLA-DR and DQ alleles are associated not just with the risk of developing lupus, but with the kinds of autoantibodies produced. Genes that control apoptosis (programmed cell death) are important in murine lupus models and likely in human lupus as well. The proteins to which the lupus patient mounts an autoantibody response are exposed on nuclear blebs during apoptosis. Genes involved in immune complex clearance (Fc-gamma receptor alleles) may predispose patients to lupus nephritis. Gene expression studies have identified an "interferon signature"—a group of genes regulated by interferon-—in the majority of SLE patients. The genetic predisposition to SLE is not overwhelming. Only 10% of patients have a first-degree relative with SLE, and SLE develops in only 2% of children who have an afflicted parent.

Environmental factors play a role not only in the onset of SLE but also in triggering the "flares" (relapses). The most recognized environmental trigger is ultraviolet light exposure. Ultraviolet (UV) light significantly by UV - B can trigger photosensitive rashes, and more rarely, systemic flares. SLE patients are more likely than controls to have drug allergies, especially to sulfonamide antibiotics. Infection with Epstein-Barr virus has been strongly associated with SLE in a multicase family registry. Hormonal factors are obviously important, given the female predominance of SLE and the usual onset of SLE after puberty. In clinical trials, hormone

replacement therapy has been associated with increased flares in SLE, but oral contraceptives did not. Pregnancy is associated with SLE flares in some, but not all, studies. Elevation of prolactin may be associated with activity of SLE.

The activity of SLE follows several patterns. The classic pattern, the flare pattern, is characterized by a relapsing-remitting course. However, an equal number of SLE patients have a pattern of continuously active disease. Only a minority of patients have long periods of disease quiescence. The antimalarial drug hydroxychloroquine, which is widely used for cutaneous lupus and lupus arthritis, reduces future flares if patients continue to take it. Over half of SLE patients have acquired permanent damage in one or more organ systems. Although damage, such as renal failure and interstitial pulmonary fibrosis, can occur from SLE itself, immunosuppressive therapy also contributes to certain damage. For example, long-term prednisone therapy may cause osteoporotic fractures, osteonecrosis, cataracts and glaucoma.

Survival of SLE patients peaked at about 80% at 10 years after diagnosis in the 1980s. The Centers for Disease Control and Prevention reported in 2002 that mortality in young women had actually increased. The major cause of death in SLE is accelerated atherosclerosis. Although SLE itself can damage the endothelial surface of the coronary arteries, part of the atherosclerotic process results from elevated levels of traditional cardiovascular risk factors, including hypertension, dyslipidemia, obesity, and homocysteine levels. Prednisolone increases the patient's weight, blood pressure, glucose, and lipid levels. SLE nephritis can lead to

hypertension and dyslipidemia. Renal insufficiency can increase serum homocysteine levels.

The description of a bimodal mortality pattern in SLE patients by **Urowitz et al**. in 1976 was an instrumental step toward identifying their increased risk of premature atheromatous cardiovascular disease (CVD). Patients who succumbed to lupus early in the disease course were noted to die most often from complications of disease activity (e.g., organ failure) or therapy. Patients who died later often had quiescent disease, and died from CV events. These findings were substantiated by an autopsy series reported by **Bulkey et al.** in which the majority of a cohort of young women with a mean age of 35 years had significant obstructive atherosclerotic disease of at least one major coronary artery (14). As survival has improved from better means of disease detection and treatment, atherosclerotic CVD has emerged as a significant cause of morbidity and mortality in SLE. The prevalence of cardiovascular and cerebrovascular events in SLE ranges from 6% to 26% **(15, 16, 17, 18, 19, 20, 21, 22, 23 & 24)**. Ischemic cerebrovascular events (stroke/transient ischemic attack) have been reported in 10% to 26%, **(17, 18, 22, 23 & 24)**, whereas MI and angina have been reported in 6% to 11% of SLE patients **(15, 16, 19, 20, 21, 22, 23 & 24)**.

Not surprisingly, autopsy studies as well as the examination of surrogate markers of coronary atherosclerosis in SLE suggest that the prevalence of subclinical atherosclerosis is higher than overt events **(14, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 & 36)**. Autopsy studies reveal atherosclerotic disease of the coronary arteries in SLE in 22% to 54% of cases **(14, 25 & 26)**. Noninvasive studies

including vascular ultrasound, electron beam tomography, and myocardial perfusion studies demonstrate atherosclerotic vascular disease in 17% to 40% of SLE patients **(27, 28, 29, 30, 31, 32, 33, 34, 35 & 36)**. In a cohort of SLE women with a mean age of 44.9 years, 40% were found to have focal carotid plaque as measured by ultrasound **(27)**. Another study of 75 SLE women with a mean age of 38.8 years demonstrated that 28% had coronary artery calcifications **(31)**. **Roman et al**. found carotid plaque in 37% of SLE patients, compared with a prevalence of 15% in age, sex, and race-matched controls **(32)**.

A striking feature of this comorbid condition of SLE is its predilection for premenopausal women. **Ward** evaluated rates of hospitalization for cardiovascular events in a cohort of SLE women compared to a control group **(37)**. Younger SLE women (age 18 to 44) were 2.27 times more likely to be hospitalized with MI and 3.8 times more for congestive heart failure than controls. In the middle-aged women (45 to 64 years), the frequency of hospitalization for heart failure was just 1.39 times higher, and the frequency for MI hospitalization did not differ significantly from that of controls. A study comparing SLE women with age-similar women from the Framingham offspring cohort demonstrated a 50-fold increased risk of MI in the SLE women between 35 and 44 years of age **(38)**. In sharp contrast to women in the general population, where the risk of atherosclerotic CV events is highest after menopause, the mean age at the first event in SLE women was earlier in their life **(39)**.

Traditional risk factors for CVD occur frequently in SLE, both as a consequence of disease activity and treatment. The presence of subclinical inflammation in the general population has been demonstrated to correlate with the development of a number of traditional risk factors, including insulin resistance, visceral adiposity and hypertension **(40, 41 & 42)**. It is possible that the sustained systemic inflammation and immune activation in SLE has similar influences on the development of CV risk factors. The Toronto Risk Factor Study compared 250 SLE patients with 250 controls and found that SLE patients had a higher number of CV risk factors per patient as well as a higher prevalence of diabetes, hypertension, and elevated levels of low-density lipoproteins, triglycerides, and homocysteine **(43)**. Additionally, both **Bruce et al. and Costenbader et al.** have demonstrated that even when CV risk factors are identified in SLE patients, they often are not adequately treated **(44,45)**. Although a large part of CVD risk in SLE is likely a result of a high prevalence of traditional CV risk factors, **Esdaile et al.** demonstrated that the presence of CV risk factors alone does not explain the increased incidence of CV events **(46)**.

Glucocorticoid therapy has been implicated in atherosclerosis in both lupus and nonlupus patients, but it is unclear whether this reflects pro-atherogenic effects of the underlying disease process or adverse metabolic effects associated with steroid use **(47, 48, 49)**.

Antiphospholipid antibodies have been considered as a contributory factor to SLE-associated atherosclerotic disease. In addition to their postulated role in arterial endothelial damage, they have been associated with renal arterial disease (both

thrombotic and stenotic), which may result in hypertension from activation of the renal-angiotensinogen-aldosterone axis from renal hypoperfusion **(50, 51)**. Although cohort studies have failed to identify an association between the presence of antiphospholipid antibodies and surrogate markers of coronary atherosclerosis, there is a strong association between surrogate markers of CVD and CV events with hypertension with in SLE **(22, 52 and 53)**. The importance of this association becomes apparent when more closely evaluated.

Cardiovascular disease and atherosclerosis are a common cause of morbidity and mortality in various SLE cohorts. Autopsy studies from the early 1980s showed severe atherosclerosis in 40% of SLE patients compared with 2% of control subjects, matched for age at the time of death. Analysis of the Swedish Hospital Discharge Register followed by linkage to the Cause of Death Register during the period 1964 to 1995 showed that SLE patients were at increased risk for death as a result of coronary heart disease or stroke (standardized mortality ratio 2.97, 95% confidence interval 2.78 to 3.16). The risk was substantially higher in the younger group of patients (20 to 39 years old; standardized mortality ratio 16, 95% confidence interval 10.4 to 23.6).

Other studies have shown that SLE patients carry an increased risk for myocardial infarction or stroke compared with the healthy population. This risk cannot be fully explained by the traditional cardiovascular disease risk factors. Atherosclerosis—defined by coronary artery calcification or carotid plaque size—also is more common in SLE patients than in healthy controls (e.g., 31% versus 9%, in subjects with an average age of 40; relative risk 9.8, 95% confidence interval 2.5

to 39), even after adjustment for possible confounding factors, and it correlates with disease activity and damage scores.

Pathogenesis of coronary atherosclerosis in SLE:

Immune Complex or Arteritis

Animal models suggest that this contributes to atherosclerosis. Vascular injury, through immune complexes, followed by exposure to atherosclerotic risk factors, can lead to atherosclerosis in animal models. Immunization of rabbits with heat-shock protein 60/65 leads to aortic intima atherosclerosis. Coronary vasculopathy and myocardial infarctions are found in murine lupus models, often in association with anticardiolipin antibody. Immune complexes from lupus sera accelerated uptake of cholesterol by smooth muscle cells. One small study in human SLE suggested that patients treated with corticosteroids had less intimal proliferation in their coronary vessels, suggesting that suppression of arteritis initially might lead to less atherosclerosis later.

Anti-phospholipid Antibodies

Anti-phospholipid antibodies could contribute to coronary artery disease through thrombosis or vasculopathy. The association of anti-phospholipid antibodies with coronary artery disease has been shown in some but not all studies. It has been found that the lupus anticoagulant is associated with angina/myocardial infarction, but not carotid plaque. Lupus anticoagulant was increased in SLE cases with cardiovascular disease vs those without. Anti-phospholipid antibodies may function also as antibodies against oxidized lipoproteins, an additional mechanism (the

"oxidative modification hypothesis") by which they might contribute to atherosclerosis. In one study, anti-oxLDL was higher in SLE cases with cardiovascular disease. However, several studies have failed to find an association of antioxLDL with arterial thrombosis, arterial disease or atherosclerosis. Finally, one of the plasma protein targets of antiphospholipid antibodies, b2 glycoprotein I, may be an important control against atherosclerosis that is perturbed by anti-phospholipid antibodies. Anti-b2 glycoprotein I accelerates uptake of oxLDL in vitro. There is also interest in lysophosphatidycholine, LPC, a high-affinity ligand for G2A, a lymphocyte expressed protein-coupled receptor. Genetic deletion of the receptor results in autoimmunity. LPC is reduced in SLE and anti-LPC has been detected.

Chronic Infection

In the non-SLE patient, there is great interest in chronic infections, including Chlamydia pneumoniae, as potential causes of atherosclerosis. Simple antibiotic regimens could conceivably eliminate these infections and reduce later coronary artery disease. Whether these chronic infections lead to accelerated atherosclerosis in SLE is currently unknown.

Coronary Artery Disease Risk Factors

Multiple studies have now proven that the risk of coronary artery disease (CAD) in SLE cannot be solely explained by traditional cardiovascular risk factors. After controlling for common risk factors at baseline, SLE patients have a relative risk of 10.1 for nonfatal MI. In a study with control subjects matched for traditional cardiovascular risk factors, SLE patients had more carotid atherosclerosis (41 vs

9%) and left ventricular hypertrophy (32 vs 5%). On average, SLE patients with coronary artery disease have one less traditional cardiovascular risk factor than a control patient. Although traditional risk factors cannot explain all atherosclerosis in SLE, they contribute significantly to the process.

Several works have found that routine coronary artery disease risk factors are very frequent in SLE patients. In fact, the average SLE patient in a cohort study has 3 or more of these routine CAD risk factors. Some of these risk factors could be due to SLE. Hypertension, for example, is more prevalent in SLE patients with renal disease. Hypertension is associated with coronary artery disease in SLE, including some, but not all, multivariate analyses.

Hyperlipidemia in SLE has two major patterns. One pattern occurs in active disease, especially in pediatric patients. These patients have low HDL cholesterol and apoprotein A1 with elevated VLDL cholesterol and triglyceride levels. Lahita and colleagues have found a similar dyslipoproteinemia in SLE patients with anti-cardiolipin antibody. However, one group has found that disease activity, rather than anti-cardiolipin, explains the reduction in HDL. There are likely defects in early cholesterol transport and VLDL metabolism associated with active SLE. The second pattern occurs in SLE patients on corticosteroids, with higher levels of triglyceride, cholesterol, and LDL cholesterol. Sustained hypercholesterolemia, rather than baseline, or intermittent elevation, is the most important predictor. A third problem identified is decreased lipolysis and chylomicron remnant removal. Lipoprotein (a) (Lpa) has been identified as a risk factor for atherosclerosis in SLE. SLE patients can make anti-lp (a). Lp (a) may rise with disease flares and be reduced by

corticosteroids. SLE patients with higher lp (a) levels also have more immune complexes containing b2 glycoprotein I.

The major cause of death in lupus nephritis patients is cardiovascular disease. Traditional cardiovascular risk factors, especially hypertension and hyperlipidemia, are increased. Tubulointerstitial lipid deposits can be found. In juvenile-onset SLE, nephrotic range proteinuria is the strongest risk factor for atherosclerosis. In a longitudinal study, three CAD risk factors, weight, cholesterol, and mean arterial pressure, were worsened by prednisone therapy. In the regression model, a 10-mg increase in prednisone led to a 5.5-lb increase in weight and an 8.9-mg% increase in total cholesterol, adjusting for all other factors known to affect these risk factors. Thus, even if the development of these CAD risk factors is directly due to SLE, prednisone treatment increases their levels.

A recent work has focused on a newly identified risk factor for cardiovascular disease, homocysteine. Homocysteine is an amino acid that has a direct toxic effect on endothelium and indirect effects, including induction of a vascular endothelial cell activator, promotion of vascular smooth muscle proliferation, and an inhibitory effect on endothelial cell growth. Hyperhomocysteinaemia has been shown to increase the risk of coronary artery disease, stroke, and carotid artery stenosis in the Physicians' Health Study and the Framingham Heart Study. In the Hopkins Lupus Cohort Study, 15% of the 337 SLE patients had elevated homocysteine. Raised homocysteine levels were significantly associated with stroke and arterial thrombosis. A retrospective study has confirmed this association.

Based on several studies the risk factor frequency can be approximated as follows:

Risk factor Frequency (%)

Family history 41

Hypertension 48

Hypercholesterolemia 56

Obesity (major) 38

Smoking—ever 56

Smoking—current 35

Sedentary lifestyle 70

Diabetes 7

Homocysteine 15

Inflammation plays a major role in the pathogenesis of atherosclerosis and myocardial infarction in the general population. The benefit of statins, for example, is mediated not just by their lipid-lowering effect, but by an anti-inflammatory effect (demonstrated by reduction in C-reactive protein) that likely stabilizes plaques. In the general population, C-reactive protein, IL-6, fibrinogen, and other markers of inflammation are predictive of atherosclerosis. In SLE, C-reactive protein was not predictive in a multivariate model nor in univariate analyses in one lupus cohort. However, in another case-control study, C-reactive protein was increased in SLE cases vs SLE controls. They did not find fibrinogen to be predictive of atherosclerosis. In one study, fibrinogen increased with duration of disease regardless of disease activity, and was increased in SLE patients with thrombosis.

Prolonged corticosteroid therapy could precipitate atherosclerosis indirectly, by increasing the levels of CAD risk factors (hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, obesity, and homocysteinemia) or directly, via vascular injury. Accelerated atherosclerosis has occurred in SLE patients without corticosteroid use, but such cases are very rare. In a 1986 review, Nashel made a strong case that the latter occurs. In animals, corticosteroids and ACTH produce vascular injury, alter vascular connective tissue and worsen experimentally induced atherosclerosis. Patients with Cushing's syndrome, before effective treatment was available, commonly developed accelerated atherosclerosis.

Flow mediated Dilatation:

The capacity of blood vessels to respond to physical and chemical stimuli in the lumen confers the ability to self-regulate tone and to adjust blood flow and distribution in response to changes in the local environment. Many blood vessels respond to an increase in flow, or more precisely shear stress, by dilating. This phenomenon is designated FMD. A principal mediator of FMD is endothelium-derived NO. The precise mechanisms for the acute detection of shear forces and subsequent signal transduction to modulate vasomotor tone are not fully understood. The endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear stress **(54, 55 & 56)**. The effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry (there are no voltage-gated calcium channels in endothelial cells). Calcium activates an enzyme, endothelial nitric oxide synthase (eNOS), and the subsequent generation of NO appears to account for FMD

(57, 58). Indeed, endothelial denudation or treatment with a nitric oxide synthase (NOS) inhibitor abolishes FMD in a variety of arterial vessels. However, it was recently shown that blood vessels from mice genetically engineered to lack the eNOS enzyme (eNOS knockout mice) still respond to shear stress by dilating. In the eNOS knockout mice, FMD seems to be mediated by endothelium-derived prostanoids, as it is blocked by indomethacin **(59)**. Thus, there is some redundancy in the system, and more than one endothelial mediator is capable of acting as the signal between endothelium and smooth muscle. It is unknown whether other mediators, such as the putative endothelium-derived hyperpolarizing factor, can cause FMD if both NO and prostanoids are deficient.

Several mechanisms may underlie the increase in NO in response to changes in shear stress. Very acute changes may be mediated by the increase in intracellular calcium that occurs when ion channels open (see the previous text). Over slightly longer time periods (minutes), shear-stress-induced phosphorylation of eNOS via a serine/threonine protein kinase, Akt/PKB, increases eNOS activity, even at low calcium concentrations, and this may be important to allow continued output of NO **(60, 61)**. In addition, other posttranslational modifications of the enzyme (myristilation or palmitoylation) or interaction with caveolin can affect intracellular localization of the enzyme and thereby alter its function. Over longer time periods (many minutes or hours), eNOS gene transcription is activated, and this can result in continued increases in NO generation if shear stress is maintained at high levels.

To assess endothelial function noninvasively, brachial arteries are scanned with high resolution ultrasound imaging, under baseline conditions (at rest) and during hyperemia induced by inflation and deflation of a sphygmomanometer cuff mostly around the forearm distal to the site to be scanned with ultrasound. The induced shear stress caused by the increased blood flow following transient ischemia induces nitric oxide (NO) release, which in turn causes local arterial vasodilatation**.** Endothelial function, defined as flow mediated dilatation (FMD), is estimated as the percentage increase in vessel diameter from baseline conditions to maximum vessel diameter during hyperemia. Impaired endothelial function of the brachial artery assessed in this manner has been reported in asymptomatic children and adults with elevated cardiovascular risk factors such as smoking **(62)**, hypercholesterolemia **(63)**, hypertension **(64)**, diabetes mellitus **(65)**, and hyperhomocysteinaemia **(66)**. Although the results of these studies are likely to be internally valid, comparison of the FMD values across studies is troublesome. FMD values vary considerably across populations, ranging from –1.9 to 19.2%.

Masoud El-Magadmi et al (67) studied 62 women with SLE (1997 revised criteria) and 38 healthy women. Demographic and risk factor data were collected. In patients, disease activity and treatment-related parameters were also assessed. Endothelial function was assessed by flow-mediated dilation (FMD) in the brachial artery in response to reactive hyperemia. Carotid intima-media thickness (IMT) and the presence of carotid plaques were also assessed in SLE patients. FMD was impaired in SLE patients (median, 3.6%; range, –6.3% to 13.7%; versus median, 6.9%; range, –6.6% to 17.8%, $P<0.01$). Using multiple regression analysis that included all subjects in which they retained all the classic CHD risk factors, they

found that systolic blood pressure (P=0.019) and SLE (P=0.017) were significantly associated with impaired FMD. Within SLE patients, IMT showed a negative correlation with percent FMD (r=–0.37, P<0.01). In stepwise multiple regression of SLE patients only, that also included SLE factors and IMT, IMT alone was independently associated with FMD (P=0.037). Two other studies have reported similar findings. In a study from Sao Paulo, **Lima et al** noted that the mean± SD FMD in SLE was 5.0±5.0% compared with 12.0±6.0% in healthy control subjects. In this study, postmenopausal women and subjects with known CHD risk factors were excluded. Piper et al found in a UK cohort that SLE women had a median FMD of 5.6 (interquartile range [IQR], 3.1% to 7.2%) compared with 8.0% (IQR, 6.3% to 9.3%) in control subjects.

Valdivielso P et al (68) analyzed endothelial function in systemic lupus erythematosus (SLE), and its relationship with disease activity and subclinical arteriosclerosis. They studied a group of 26 patients with SLE and 21 age- and sex-matched controls. None of the patients or controls had had any ischemic event. Data were recorded on medical history, anthropometrics, prior treatment and the lupus activity index (LAI). Endothelial function was quantified by flow-mediated dilatation in the brachial artery. The presence of subclinical arteriosclerosis was assessed by the average intima-media thickness (IMT) on carotid ultrasound. The patients and the controls had a similar degree of carotid IMT (0.58+/-0.08 mm vs. 0.57+/-0.07 mm, NS) and a similar prevalence of carotid plaque (27% vs. 24%, NS). However, the SLE patients had worse endothelial function than the controls (FMD 12.4+/-4.4% vs. 16.9+/-5.5%, p<0.05). This difference remained after adjusting for age, smoking, body mass index, waist circumference, total cholesterol, triglycerides,

HDL cholesterol, apolipoproteins A-1 and B100 and postmenopausal status. A significant association was found in the SLE patients between FMD and LAI (Spearman Rho -0.462, $p<0.05$). SLE-associated endothelial dysfunction was present in patients who have no prior ischemic events and with the same degree of subclinical arteriosclerosis as controls. Moreover, the endothelial dysfunction was significantly associated with the degree of disease activity. There was difference among these studies with regard to the inclusion of disease duration, comorbities & use of immunosuppressants.

Piper M K et al (70) compared thirty-six female SLE patients with 22 healthy age and sex matched controls. Endothelial dependent vasodilatation (EDD) was assessed at the brachial artery in response to shear stress. SLE patients showed significantly impaired endothelial function (median EDD 5.6%, IQR 3.1-7.2%) compared with healthy controls (median EDD 8.0%, IQR 6.3-9.3%; P = 0.001).

Zahra Seyyedbonakdar et al (69) evaluated the prevalence of vascular endothelial dysfunction and its risk factors in SLE women and then to identify its correlation with the disease activity, duration and concomitant conditions in these patients. Eighty four female SLE patients and 18 healthy young female were included in the study. The vascular endothelial function was evaluated via ultrasonographic assessment of the brachial artery diameter to determine flow mediated dilation (FMD). SLE patients had higher prevalence of impaired FMD than healthy subjects (48.8 vs. 5.5 %). But FMD impairment did not have any significant correlation with disease activity, duration, the presence of the anti-dsDNA, anticardiolipin antibodies, antiphospholipid syndrome, hypertension,

hyperlipidemia, diabetes mellitus, hypothyroidism, history of lupus nephropathy, and history of receiving cyclophosphamide pulses.

Elizabeth Turner et al (71) measured flow-mediated dilation of the brachial artery using high resolution ultrasound and the presence or absence of coronary calcification by electron beam computed tomography. Twenty patients (17 female) median age (interquartile range) 42.5 (32.0–47.5) years were studied. The median flow-mediated vasodilatation was 3.6% (1.7%–7.7%). In patients with coronary calcification (n = 6), flow-mediated dilation was 2.1% (−0.42%–3.6%) compared with 4.0% (3.5%–8.3%) in those without (p = 0.12). There was no significant relationship between flow-mediated dilation and markers of disease activity, duration of disease, and cardiovascular risk factors. Lower flow-mediated dilation was associated with duration of corticosteroid therapy.

Lai-Shan Tam et al (72) examined whether acute hyperhomocysteinaemia exacerbates endothelial and platelet dysfunction in patients with SLE. Twelve SLE patients and 15 controls were recruited. Oral methionine was used to achieve acute hyperhomocysteinaemia. Endothelial function was assessed by flow-mediated dilatation (FMD) of the brachial artery; also assessed were the levels of von Willebrand factor (vWF) and plasminogen activator inhibitor type 1 (PAI-1). Platelet activation was assessed by the levels of beta-thromboglobulin (beta-TG), fibrinogen binding, and P-selectin expression using flow cytometry. After oral methionine loading, vWF levels increased significantly, whereas FMD remained unchanged in both groups. PAI-1 increased significantly only in controls. Fibrinogen binding to platelets increased significantly only in SLE patients. Beta-TG remained

unchanged in SLE patients but increased significantly in controls. Platelet P-selectin expression did not change in either group. These results suggest that the prothrombotic tendency after acute hyperhomocysteinaemia is mediated by endothelial dysfunction and platelet activation in patients with SLE and healthy controls.

Parasar Ghosh et al (73) studied Asian Indians with SLE, to find out the prevalence and predictors of carotid intima-medial thickness (IMT) and brachial artery flow-mediated dilatation. Endothelial dysfunction was assessed by FMD in brachial artery and IMT was measured in common carotid artery in SLE patients and healthy controls. Sixty SLE patients (mean age 31±9 years) and 38 healthy controls (mean age 34±6 years) were included. The IMT was higher in SLE patients as compared to controls (0.49±0.08 mm vs. 0.39±0.05 mm, $p<0.0001$). SLE and damage were independent predictors of abnormal IMT. FMD was impaired in SLE patients compared to controls (9.97% vs. 18.97%, $p<0.00001$). None of the classical cardiovascular risk factors were predictors of FMD or abnormal IMT. Their study has shown a good negative correlation between brachial FMD and carotid IMT which have also been shown in other studies **(74).**

Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery (9).

Mary C. Corretti, MD et al has led the report submitted by the International Brachial Artery Reactivity Task Force.

Subject preparation:

Numerous factors affect flow-mediated vascular reactivity, including temperature, food, drugs and sympathetic stimuli, among others. Therefore, subjects should fast for at least 8 to 12 h before the study, and they should be studied in a quiet, temperature-controlled room. All vasoactive medications should be withheld for at least four half-lives, if possible. In addition, subjects should not exercise, should not ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.

Equipment:

Ultrasound systems must be equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler and a high-frequency vascular transducer. A linear array transducer with a minimum frequency of 7 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images with sufficient resolution for subsequent analysis. Image resolution is enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers.

Image acquisition:

The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging.

Endothelium-dependent FMD: To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff is first placed either above the antecubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume. Thereafter, arterial occlusion is created by cuff inflation to suprasystolic pressure. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation.

Studies have variably used either upper arm or forearm cuff occlusion, and there is no consensus as to which technique provides more accurate or precise information. When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm **(75, 76 & 77)**. This may be

due to a greater flow stimulus resulting from recruitment of more resistance vessels or possibly to direct effects of ischemia on the brachial artery. However, upper-arm occlusion is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue. The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 s to 5 min. The change in diameter is similar after 5 and 10 min of occlusion; therefore, the more easily tolerated 5-min occlusion is typically used. Also, FMD may be studied in the radial, axillary and superficial femoral arteries. Notable caveats are that arteries smaller than 2.5 mm in diameter are difficult to measure and vasodilation is generally less difficult to perceive in vessels larger than 5.0 mm in diameter **(78, 79 & 80)**.

Anatomic landmarks:

The diameter of the brachial artery should be measured from cross sectional images in which the lumen-intima interface is visualized on the near (anterior) and far (posterior) walls. These boundaries are best visualized when the angle of insonation is perpendicular. Thus, clear visualization of both the near and far wall lumen-intima boundaries indicates that the imaging plane is bisecting the vessel and diameters measured from these images likely reflect the true diameter. Once the image for analysis is chosen, the boundaries for diameter measurements are identified manually with electronic calipers or automatically using edge-detection software. The variability of the diameter measurement is greatest when it is determined from a point-to-point measurement of a single frame, and least when

there is an average derived from multiple diameter measurements determined along a segment of the vessel.

The diameter measurement along a longitudinal segment of vessel is dependent upon the alignment of the image. Skew occurs when the artery is not completely bisected by the plane of the ultrasound beam. With slight skew, the maximal diameter measured is constant, and thus yields a more accurate measurement. Some edge-detection programs can account for skew from transducer angulation **(79, 81)**.

Timing of FMD:

Flow-mediated vasodilation is an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased shear stress. Increased flow, and thereby increased shear stress, through the brachial artery occurs during post occlusive reactive hyperemia. Several studies have suggested that the maximal increase in diameter occur approximately 60 s after release of the occlusive cuff, or 45 to 60 s after peak reactive hyperemic blood flow **(80, 82)**. The increase in diameter at this time is prevented by the NOS inhibitor NG-monomethyl-L-arginine, indicating that it is an endothelium-dependent process mediated by NO **(83, 84)**. Other measures of vasodilator response include time to maximum response **(85)**, duration of the vasodilator response and the area under the dilation curve.

Characterizing FMD:

Flow-mediated vasodilation is typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter **(86)**.

Evaluating precision of the technique:

Intraobserver and interobserver variability in image acquisition and analysis should be established and periodically reassessed for each condition, including baseline, reactive hyperemia and NTG administration. The highest reproducibility is likely to be shown over a short interval, during which the individual vasodilator response is unlikely to have changed owing to environmental or other influences. This can be accomplished by taking two measurements on the same day within a 10- to 15-min interval, or on separate days in otherwise identical circumstances. Longitudinal studies in which interventions over weeks to months are tested require that reproducibility measurements be performed at longer intervals. The image analysis and measurement of the vasodilator response from repeated studies should be performed by an individual who is blinded as to sequence. Measurement variability is assessed, typically, by a designated core laboratory for multicenter trials, prior to site certification and periodically thereafter to analyze for temporal drifts. Assessment of FMD of the brachial artery in clinical trials has increased because of its seeming ease of use, efficiency and noninvasive nature. Owing to the biological and technical variability of the measurement, several caveats should be considered when planning a clinical trial where FMD is the end point of interest. These include study design, sample size and uniform technique.

Study design:

Recent studies have reported on the effect of pharmacologic or physiologic interventions on FMD of the brachial artery. These include both acute **(87, 88, 89)**

and longer-term intervention trials **(90, 91&92)**. Both parallel-group and crossover designs have been successfully employed.

Sample size.

Typically, significant improvement in FMD can be seen with 20 to 30 patients in a crossover design study and 40 to 60 patients in a parallel-group design study. In studies of this size, the minimal statistically significant improvement that can be detected with intervention is an absolute change in FMD of 1.5% to 2%. The sample size depends greatly on the variance of repeated measurement in the control group in a particular vascular laboratory.

With intervention trials, an important parameter to report is the time-dependent reproducibility of FMD. For example, in the placebo group, the pretreatment and post intervention FMD measures are usually reported, and often are very similar. However, if the mean difference between the two measurements for each patient is quite high, it indicates that the variance of the technique might limit interpretation of the study results. An acceptable reproducibility is a mean difference of 2% to 3% in FMD over time (on a baseline vasodilation of about 10%) **(93)**. This value has not been readily available in published trials.

Methodology:

As discussed above, several techniques have been employed to measure FMD **(94, 95)**. Laboratories should select the method that gives them the most reproducible results, and for multicenter studies, the same scanning protocol should be employed at all sites. For studies employing repeated measurements following

intervention, FMD might change as a result of the intervention. However, FMD could also be affected by a change in the hyperemic stimulus. Therefore, the flow stimulus should be consistent. Otherwise, any change in FMD of the conduit artery may be related to changes in flow (indirectly mediated by changes in the microcirculation) rather than improvement of endothelial function of the conduit vessel per se.

Ultrasound assessment of brachial artery FMD has yielded important information about vascular function in health and disease, yet several new approaches and technological advances have emerged. Most prior studies examined FMD at a single time point, typically 1 min after cuff release. This practice evolved from the observations that the maximal dilator response occurs at approximately 1 min in healthy subjects (96) and that the necessity for manual acquisition and measurement placed a practical limit on the number of image frames that could be analyzed.

Commercially available technology now makes it possible to acquire multiple images of the brachial artery automatically using the ECG signal as a trigger and to measure arterial diameter automatically using computer-based edge-detection techniques. This approach allows investigators to examine the entire time course of brachial dilation in response to reactive hyperemia, the true peak response, the time to peak and the overall duration of FMD as discussed in the previous text. The time course and extent of brachial expansion within a single cardiac cycle, possibly reflecting vessel compliance, can be examined. In the carotid artery,

compliance has been shown to correlate with cardiovascular risk **(97)**. About 70% of the dilation observed 1 min after cuff release is attributable to NO synthesis **(98, 99)**.

AIM

AIM OF THE STUDY

1) To evaluate endothelial function and to assess the extent of dysfunction in newly diagnosed SLE patients by using the measures of flow mediated dilatation of the brachial artery and carotid intima media thickness.

2) To study the correlation of flow mediated dilatation with carotid intima medial thickness.

3) To study the relationship between endothelial dysfunction and clinical characteristics of SLE.

MATERIALS AND METHODS

MATERIALS AND METHODS

Inclusion Criteria:

1) Newly diagnosed SLE patients by ACR criteria of age 16 yrs and above.

Exclusion Criteria:

1) Patients with co morbidities like hypertension, diabetes mellitus and hyperlipidemia.

2) Patients with history of cardiovascular disease (angina, myocardial infarction, congestive cardiac failure).

3) Patients with renal failure (creatinine >3 mg/dl or creatinine clearance <30 ml/min).

4) Patients who were on long term medications like prednisolone, other immunosuppressants & statins prior to our evaluation.

5) Patients with clinical evidence of upper limb vascular insufficiency in the form of pre gangrene or gangrene.

6) Patients with overlap syndrome.

7) Infections in the previous four weeks.

8) Pregnancy or lactation in the previous 6 weeks.

Subjects:

Patients were recruited from the rheumatology outpatient clinic and wards of Government General Hospital, Chennai, during the period February 2009- February 2010. Fifty eligible patients older than 16 years of age were enrolled. They fulfilled at least four classification criteria for systemic lupus erythematosus by 1997 ACR revised criteria, with no known preexisting cardiovascular disease; and were willing to undergo measurement of flow-mediated dilation.

Healthy control subjects were recruited from the clinically normal secretarial and support staff as well as from friends of patients. All subjects gave written informed consent to take part in this study, which was approved by the ethical committee.

Clinical and Laboratory Assessment:

All subjects had a detailed history and examination to look for traditional cardiovascular disease risk factors, vasculitis, Raynaud's phenomenon, and secondary antiphospholipid syndrome. In patients, disease activity and cumulative damage were assessed by the SLE Disease Activity Index (SLEDAI) and the Systemic Lupus International Collaborating Clinics (SLICC) respectively. Laboratory evaluations include complete blood count, ESR (Westergren's method), liver function test, renal function test, fasting blood sugar and lipid profile. Immunological assay were CRP (Latex agglutination method); ANA (CalBiotech Inc ANA Screen ELISA kit); Anti dsDNA (Wampole laboratories dsDNA ELISA test); Anti cardiolipin antibody (CalBiotech cardiolipin IgG & IgM ELISA kit) &

C3, C4 complement levels (Nephelometry). The lupus anticoagulant study was done with the dilute Russell viper venom test & activated partial thromboplastin time; were also assayed.

ELISA results were interpreted as follows:

ANA

< 0.9 – Negative.

0.9- 1.1 - Borderline positive.

> 1.1 - Positive.

Anti dsDNA

< 0.9 - Negative

0.91 – 1.09 – Equivocal

> 1.1 – Positive.

ACL IgG

< 10 GPL– Negative

10- 15GPL – Borderline positive.

15- 80 GPL– Moderate positive.

>80 GPL– High positive.

ACL IgM

< 15 MPL – Negative

15- 20 – Borderline positive

21- 80 – Moderate positive

> 80 – High positive.

Complement Assay (Nephelometry)

Normal range:

C3 - 88 – 201 mg/dl.

C4 – 16 – 47 mg/dl.

All patients had Electrocardiogram, chest X ray, ultrasound abdomen and an Echocardiogram done by a cardiologist.

Assessment of Endothelial Function:

Endothelial function was assessed with high-resolution B-mode Doppler (Siemens Alterces with a 7.5 MHz linear-array transducer **Photo (A))** examination of the brachial artery using the protocol described as in the guidelines cited above **(9)**. We measured flow-mediated dilation (FMD) in response to reactive hyperemia. All subjects were studied between 8 and 11 AM after a12-hour overnight fast. They were asked not to smoke on the morning of study and to avoid alcohol for 48 hours. The brachial artery was scanned 10 cm above the antecubital fossa **(Photo B)**. Distance measured was from anterior to posterior M lines (media-adventitia interface), and every measurement was taken by sonologist blinded to cases and controls. Then, ischemia was induced by inflating the pneumatic cuff to a pressure 50 mmHg above systolic one, in order to obliterate the brachial artery. After 5 minutes, the cuff was deflated and the diameter was measured after 60 seconds post-deflation (Df).

FMD is calculated as follows: 100 %x [(post deflation diameter–resting diameter)/resting diameter]. To assess reproducibility of our technique, we looked at the reliability of reading scans on 2 separate occasions by a single blinded observer. For this, 15 scans from patients or control subjects were chosen at random. The intraclass correlation coefficients for resting diameter and FMD were 0.93 (95% CI, 0.56 to 0.95) and 0.82 (95% CI, 0.30 to 0.97), respectively.

A)

(B)

Carotid Artery Intima -Media Thickness and Plaque:

Patients and control subjects also had the intima-media thickness (IMT) of their carotid artery measured using high-resolution B-mode Doppler (Siemens Alterces with a 3-15 MHz linear-array transducer). The common carotid artery was scanned longitudinally, and the IMT measurement was taken in the proximal part of the common carotid artery, 1 cm proximal to the carotid bulb as the maximum distance between the intima-lumen and adventitia-media interfaces in areas without carotid plaque **(100)**. IMT was determined as the average of 6 measurements, 3 each from the left and right common carotid arteries. This is shown in the **Photo E**.

We also noted the presence or absence of carotid plaques, with plaque being defined using the criteria described by **Li et al (101)**. The intraclass correlation coefficient for IMT measurements, assessed in 15 subjects on 2 separate occasions 2 weeks apart, was 0.92 (95% CI, 0.84 to 1.00).

Statistical Analysis

The statistical analysis was performed using the SPSS (version 17.0).

Results are presented as mean ± SD, except for frequencies, which are expressed as percentages.

Unpaired student's t- test has been used for comparing the FMD, carotid IMT and other features of the study and control group.

$p < 0.05$ was considered statistically significant.

Pearson's test has been used for studying the correlation between flow mediated dilatation and variables.

RESULTS

RESULTS

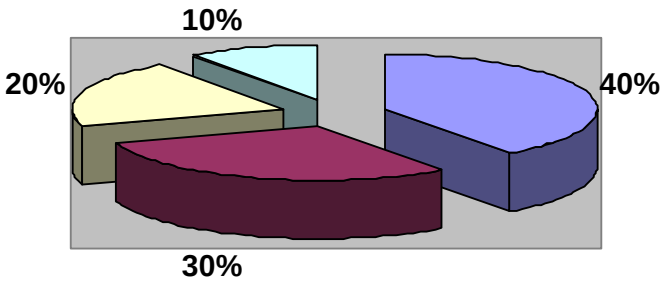
The demographic, clinical and investigation features of the studied groups were as follows:

TABLE 1: Characteristics of the group

Parameter	Patient (n=50)	Control (n=50)	P value
Male/Females	3/47	3/47	1.00
Mean Age (yrs)	23 ± 5.41	23 ± 5.41	1.00
BMI (kg/m2)	19.6 ± 1.77	20 ± 1.71	0.31
Mean duration of symptoms (months)	7.3	0	-
Systolic BP (mmHg)	112 ± 8.03	111 ± 7.7	0.59
Diastolic BP (mmHg)	79.6 ± 5.83	79.2 ± 5.52	0.75
Total Cholesterol (mg/dl)	169 ± 23.4	175 ± 19.7	0.13
Triglycerides (mg/dl)	114 ± 11.9	113 ± 10.2	0.56
HDL (mg/dl)	43.6 ± 3.5	42.9 ± 2.51	0.20
LDL (mg/dl)	108 ± 10.3	105 ± 8.61	0.09

There were 47 females and 3 males, who were age and sex adjusted in the patient and control group. The mean duration of disease according to the onset of symptoms, was 7.3 months with a minimum duration of one month to a maximum of 14 months. There was no statistically significant difference with regard to the blood pressure and fasting lipid profile between both the groups.

Age distribution of the population



Clinical characteristics of the SLE patients:

The patient group had the following clinical features in the proportion given: mucocutaneous – 88% (n=44); constitutional – 86% (n=43); musculoskeletal – 41% (n=82%); serositis – 22% (n=11); hematological – 6% (n=12%); neuropsychiatric – 8% (n=4); renal – 2% (n=1); Raynaud's – 0. The mean SLEDAI score was 11.6.The median SLEDAI score was 11 (range, 3 to 29). The median SLICC was 0 (range 0 to 1). Since we had chosen patients at diagnosis, and by excluding the patients who had been on immunosuppressants prior to our evaluation, we had a relatively naïve population who had not been on specific disease modifying drugs.

On evaluation, the patient population had the following immunological features.

TABLE 2: Immunological profile the patient group was as follows:

Parameter	Patient group (n=50)
ANA	100% (n=50)
Anti dsDNA	78% (n=39)
Low C3/C4 assay	36% (n=18)
ACL IgG/IgM	16% (n=8)
LAC	10% (n=5)

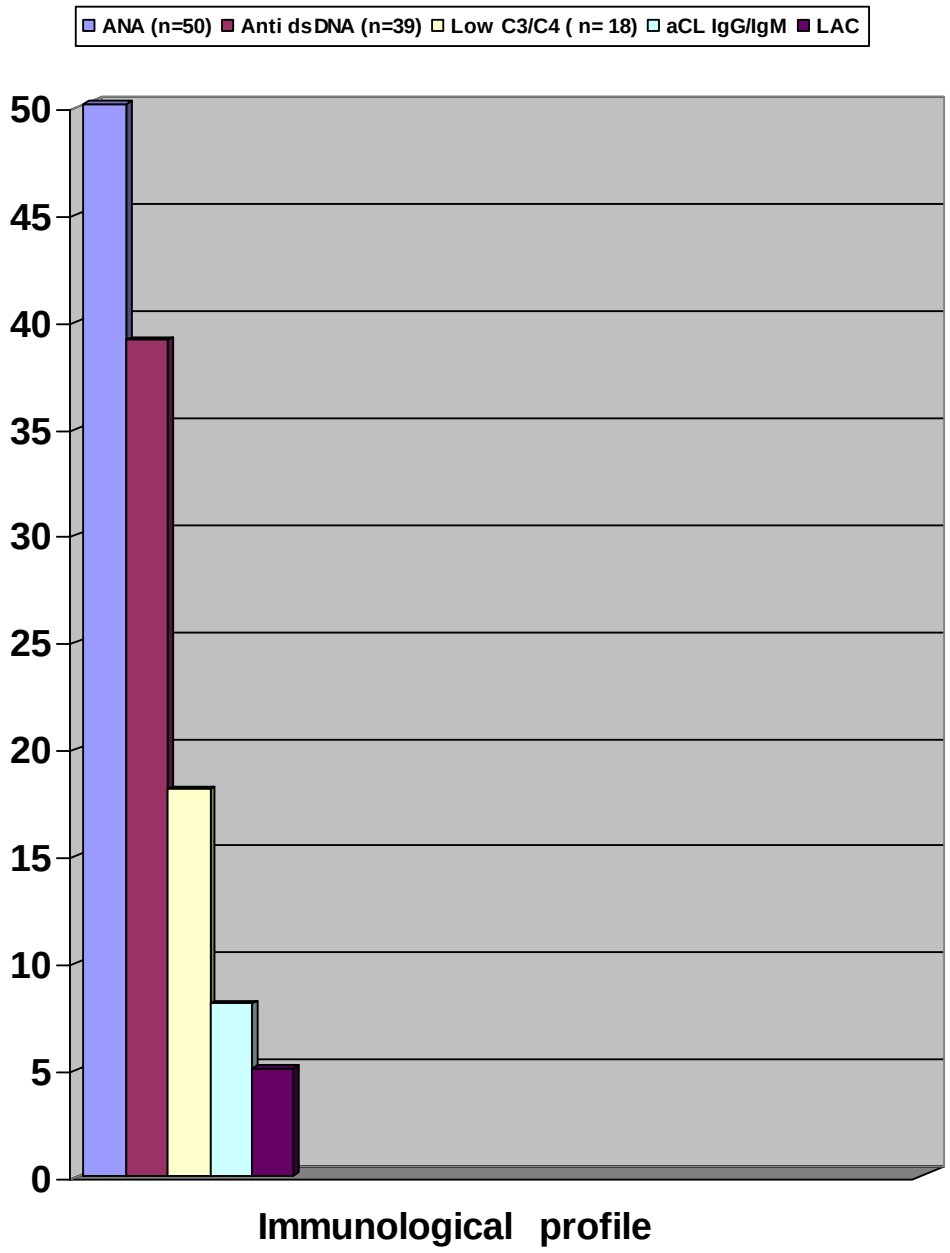
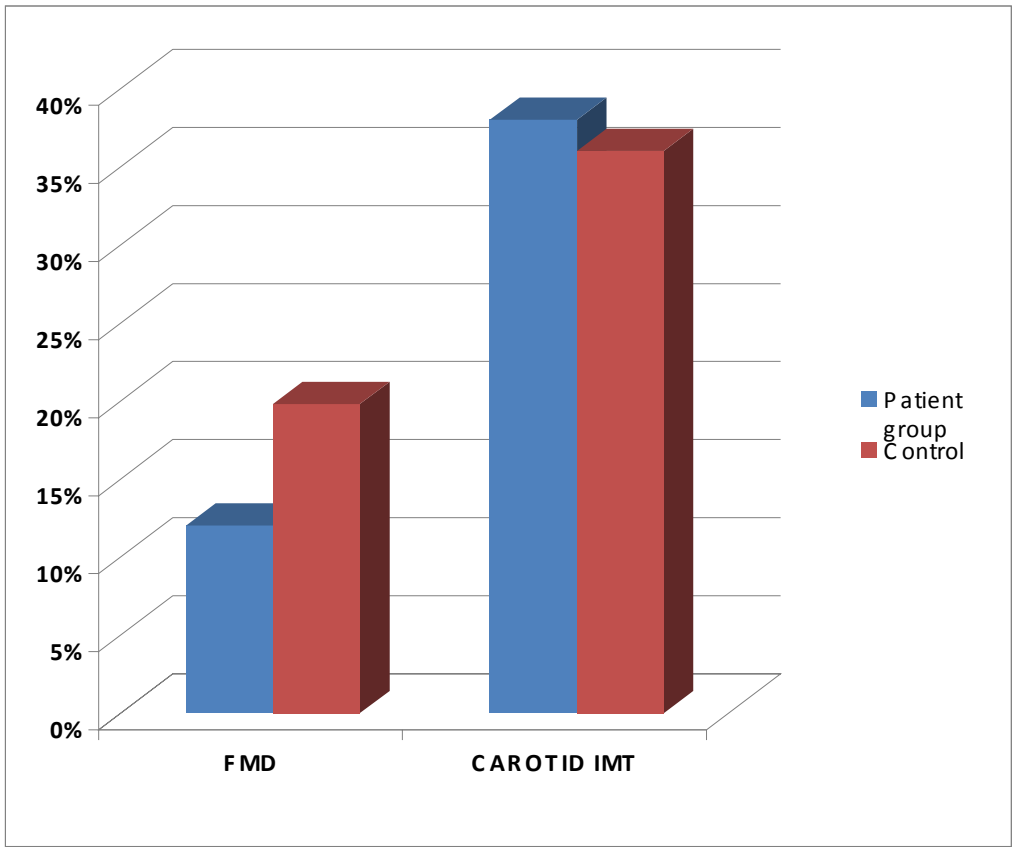

ANA (n=50)
Anti dsDNA (n=39)
Low C3/C4 (n= 18)
aCL IgG/IgM
LAC
50
45
40
35
30
25
20
15
10
5
0
Immunological profile

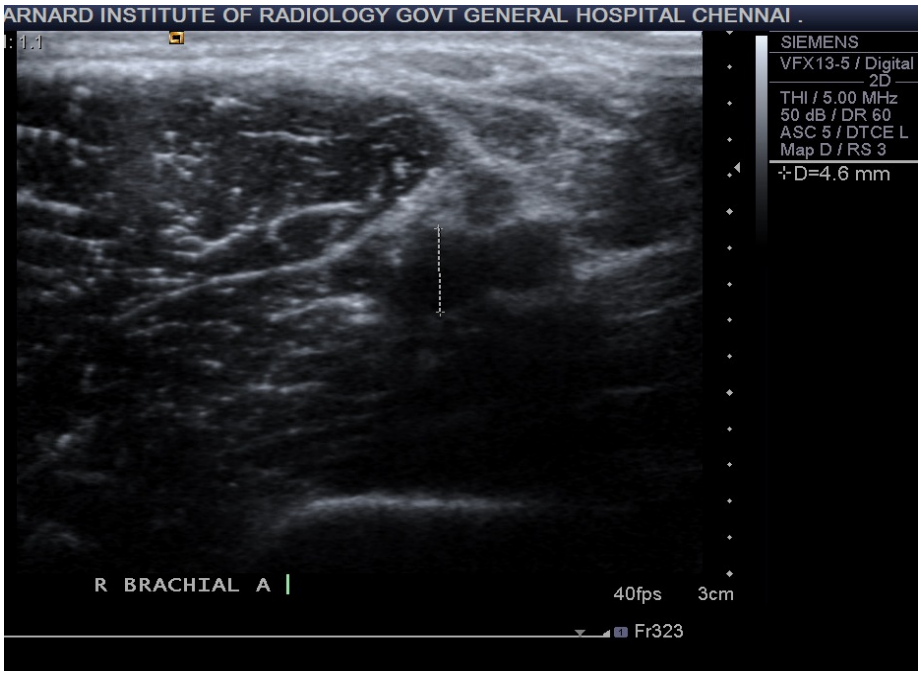
TABLE 3: Study characteristics in the patient and control group

Parameter	Patient group	Control group	p value
Baseline Diameter (mm)	3.64± 0.40	3.67 ± 0.34	0.64
FMD (%)	12 ± 3.92	19.8 ± 2.27	< 0.001
Carotid IMT (mm)	0.38	0.36	0.09

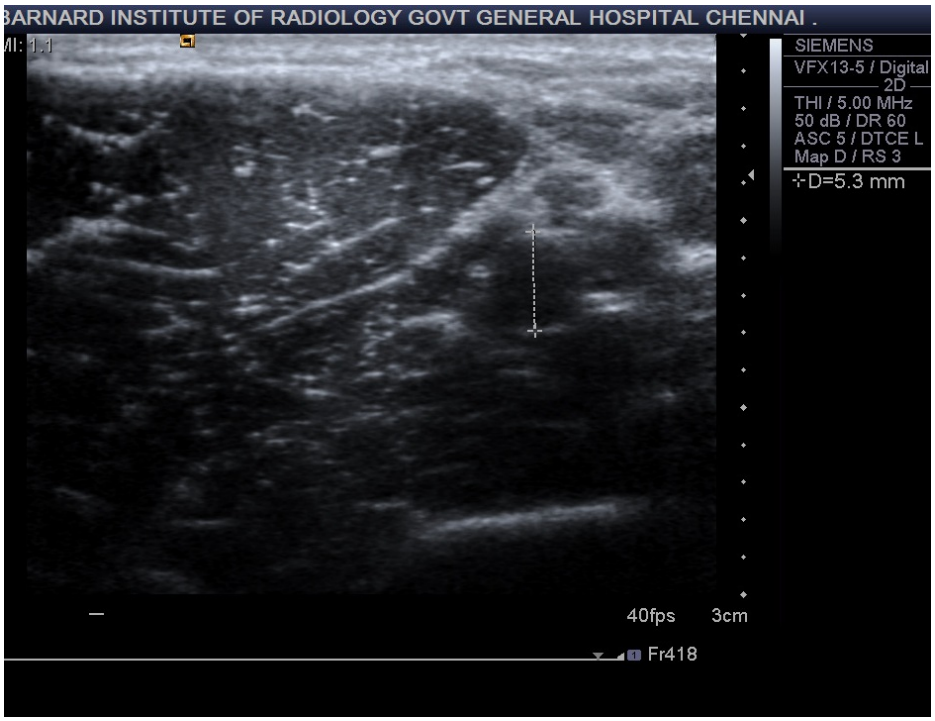
The patients and the controls had a similar degree of baseline diameter of the brachial artery (3.64 ± 0.40 vs. 3.67 ± 0.34 mm, p = 0.64) and carotid IMT (0.38 ± 0.08 vs. 0.36 ± 0.06, p value 0.09). However, the SLE patients had worse endothelial function than the controls (FMD 12 ± 3.92% vs. 19.8 ± 2.27%, p<0.001). The endothelial function, assessed by vascular ultrasonography on brachial artery, is shown (Baseline **Photo (C)** & post dilatation **Photo (D).** The carotid IMT measurement is shown in **Photo (E).**


40%
35%
30%
25%
20%
15%
10%
5%
0%
FMD
CAROTID IMT
Patient group
Control

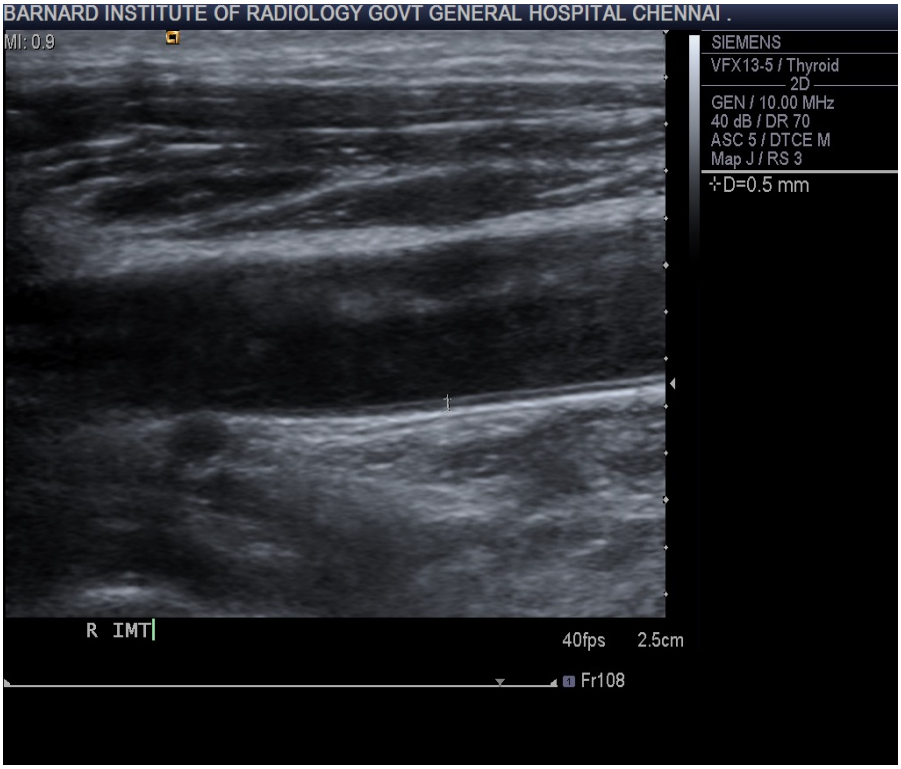
C)


ARNARD INSTITUTE OF RADIOLOGY GOVT GENERAL HOSPITAL CHENNAI .
SIEMENS
VFX13-5 / Digital
2D
THI / 5.00 MHz
50 dB / DR 60
ASC 5 / DTCE L
Map D / RS 3
D=4.6 mm
R BRACHIAL A
40fps
3cm
Fr323

(D)


ARNARD INSTITUTE OF RADIOLOGY GOVT GENERAL HOSPITAL CHENNAI .
SIEMENS
VFX13-5 / Digital
2D
THI / 5.00 MHz
50 dB / DR 60
ASC 5 / DTCE L
Map D / RS 3
D=5.3 mm
40fps
3cm
Fr418

(E)


BARNARD INSTITUTE OF RADIOLOGY GOVT GENERAL HOSPITAL CHENNAI .
MI: 0.9
SIEMENS
VFX13-5 / Thyroid
2D
GEN / 10.00 MHz
40 dB / DR 70
ASC 5 / DTCE M
Map J / RS 3
D=0.5 mm
R IMT
40fps
2.5cm
Fr108

Correlations between FMD and SLE parameters:

The correlations between FMD and biological and immunological parameters are shown in Table 4.

TABLE 4: Parameter Correlation coefficient

Disease duration	**r = 0.0998**	**p = 0.49**
Carotid IMT	**r = – 0.1993**	**p = 0.16**
SLEDAI	**r = – 0.5678**	**p = <0.01**
C3	**r = 0.9101**	**p = < 0.01**
Anti dsDNA antibodies	**r = – 0.8658**	**p = <0.01**
Total cholesterol	**r = 0.0239**	**p = 0.86**
ACL IgG	**r = 0.2388**	**p = 0.09**
ACL IgM	**r = 0.2149**	**p = 0.13**

The statistical analysis showed an inverse correlation between FMD and antidsDNA and SLEDAI which is significant statistically. Similarly there is strong positive correlation between FMD and C3. But, correlation does not imply causation.

The size of any correlation generally evaluates as follows:

Value of r	Qualitative Description of the Strength
–1	perfect negative
(–1, –0.75)	strong negative
(–0.75, –0.5)	moderate negative
(–0.5, –0.25)	weak negative
(–0.25, 0.25)	no linear association
(0.25, 0.5)	weak positive
(0.5, 0.75)	moderate positive
(0.75, 1)	strong positive
1	perfect positive

DISCUSSION

DISCUSSION

The patients with SLE have a high incidence of atherosclerosis with its main consequence: coronary artery disease. Epidemiological studies have shown that SLE women aged 35 – 44 years were over 50 times more likely to develop myocardial infarction than women of similar age from general population **(3)**. Anatomo-pathological investigations have revealed that the SLE patients were prone to develop a premature atherosclerosis **(102)**. The increased risk of atherosclerosis is not exclusively related to traditional risk factors alone **(103)**. In the last years, SLE itself appeared like an independent risk factor for atherosclerosis, acting through autoimmune vascular injury **(6)**. In patients with systemic lupus erythematosus, atherosclerosis has a long period of subclinical evolution. The first reversible step in the atherogenesis process is represented by the endothelial dysfunction **(104)**.

Endothelial dysfunction is well described entity in connective tissue diseases, leading to accelerated atherosclerosis and associated morbidity and early mortality. There are several associated factors like body mass index, co morbidities, immunosuppressants, dyslipidemia, hypertension which could contribute to the endothelial dysfunction. Our study was designed to first identify the prevalence of this in our patients and more importantly attempt to delineate the disease contribution from all associated factors. In this regard, we have chosen newly diagnosed cases who have not been on treatment and have excluded other associated factors like hypertension, dyslipidemia & diabetes mellitus. We could thereby to an extent, isolate the disease related factor and attribute the endothelial dysfunction to it.

Endothelial dysfunction appears when the normal functions of the endothelial cells (control of vascular tone and blood pressure, regulation of leucocytes traffic from the blood to the tissues, and platelet adhesion and aggregation, maintenance of the balance between blood coagulation and fibrinolysis, control of growth, development and differentiation of the vessel wall cells) are lost or dysregulated **(105)**. A non-invasive method for the assessment of endothelial dysfunction is represented by flow mediated vasodilation (endothelium dependent dilation) **(106)**. In our study, FMD in SLE patients was significantly lower than in control subjects (FMD 12±3.92% vs. 19.8±2.27%, p<0.001). There are several studies with similar results, starting with the first study, performed by **Lima *et al*. (107)**, who showed that SLE patients presented lower FMD than sex and age-matched controls (5.0±5.0% vs. 12.0± 6.0%) , even in subjects without traditional cardiovascular risk factors **(70)**. **Tani *et al*. (16)** identified the same pattern of FMD in SLE patients as well as by **Valdivielso et al (68)** (FMD 12.4+/-4.4% vs. 16.9+/-5.5%, p<0.05). The reduced values of FMD in patients with SLE were found by **Piper and Turner** in their studies, too **(67, 71)**. Our study concurred with the FMD results of the other indian study done by **Parasar Ghosh et al (73)** which was impaired in SLE patients compared to controls (9.97% vs. 18.97%, $p<0.00001$).

The Carotid IMT values showed no significant difference in our study and control population (0.38 ± 0.08 vs. 0.36 ± 0.06, p value 0.09). Similar results were obtained in the study done by **Valdivielso et al (68)** (0.58+/-0.08 mm vs. 0.57+/-0.07 mm, NS). **Parasar Ghosh et al (73)** have reported higher carotid IMT in SLE patients as compared to controls (0.49±0.08 mm vs. 0.39±0.05 mm, $p<0.0001$) and

had shown a good negative correlation between brachial FMD and carotid IMT, which was not the case in our study. **Masoud El-Magadmi et al (67) & Zahra Seyyedbonakdar et al (69)** also observed a negative correlation with percent FMD in their studies. The reason for this difference could be that in early stages of the disease to which our patients belonged to, identification of a functional abnormality (FMD) would have preceded the structural changes that occur in the carotid arteries in the form of intima medial thickness that occurs on a later stage. Moreover the other factors which would contribute to subclinical atherosclerosis like comorbities, immunosuppressants were not excluded in their studies as against ours.

We found a moderate negative correlation between FMD and SLEDAI ($r = -0.7321$, $p < 0.001$). This correlation between FMD and the disease activity was identified in other studies **(96, 108 & 109)**. **Valdivielso et al (68)** also have reported significant correlation between FMD and LAI (Lupus Activity Index). AntidsDNA had a strong negative correlation, while C3 complement assay had strong positive correlation with flow mediated dilatation. There was no linear correlation between other factors like disease duration, cholesterol, anticardiolipin antibodies.

When comparing our study with the other indian study done by **Parasar Ghosh et al (73)** regarding other factors, our study population has less disease duration (mean 7.3 months) when compared to their patient population (mean 60 months). Similarly their patient population had co morbidities (hypertension, diabetes mellitus, dyslipidemia), and had been exposed to several immunosuppressants. These factors have their contribution in the endothelial dysfunction.

FMD using vascular ultrasonography on brachial artery represents a useful, non-invasive method for the assessment of the endothelial dysfunction. Reactive hyperemia produces a shear stress stimulus that induces the normal endothelium to release nitric oxide (NO), which acts as a vasodilator. Impaired endothelial function is associated with a reduced release of NO and a lower vasodilation **(110)**. The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is under explored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature.

Ongoing studies in several large populations, including the Framingham Heart Study and the Cardiovascular Health Study, shall determine whether endothelial dysfunction in the brachial artery will identify patients at risk for developing coronary artery disease, cerebral vascular disease and/or peripheral vascular disease. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention.

Numerous studies have demonstrated that brachial artery reactivity improves with risk factor modification and treatment with drugs known to reduce cardiovascular risk. It remains unknown whether an improvement in endothelial function directly translates into improved outcome. In the future, however, practitioners may use brachial artery FMD to assess response to drug therapy and to individualize patient risk factor modification programs. Further studies are needed to determine whether the methodology is sufficiently reproducible and whether

biological variability is sufficiently low to make assessment of FMD a clinically useful measure of cardiovascular risk on an individual or group basis. To that end, the methodology will need to mature, with formal opportunities for training, certification and continuing medical education, as currently exist for other cardiovascular testing modalities.

FUTURE DIRECTIONS

Ultrasound assessment of brachial artery FMD has yielded important information about vascular function in health and disease, yet several new approaches and technological advances have emerged. Most prior studies examined FMD at a single time point, typically 1 min after cuff release. This practice evolved from the observations that the maximal dilator response occurs at approximately 1 min in healthy subjects **(111)** and that the necessity for manual acquisition and measurement placed a practical limit on the number of image frames that could be analyzed. Commercially available technology now makes it possible to acquire multiple images of the brachial artery automatically using the ECG signal as a trigger and to measure arterial diameter automatically using computer-based edge detection techniques. This approach allows investigators to examine the entire time course of brachial dilation in response to reactive hyperemia , the true peak response, the time to peak and the overall duration of FMD as discussed in the previous text. The time course and extent of brachial expansion within a single cardiac cycle, possibly reflecting vessel compliance, can be examined. In the carotid artery, compliance has been shown to correlate with cardio- vascular risk **(112)**. About 70% of the dilation observed 1 min after cuff release is attributable to NO synthesis.

Further studies are needed to evaluate other vasoactive mechanisms and to determine whether various disease states influence the kinetics and/or extent of FMD. Careful examination of the vasodilator response to NTG provides another potential avenue for investigation. Although most studies have detected little effect

of disease states on this response, there is evidence that cardiovascular risk factors might impair the vasodilator response to NTG, especially when a dose-response curve is measured. These findings are consistent with experimental studies demonstrating that inactivation of NO by reactive oxygen species is an important mechanism of vascular dysfunction. Further information about the causes of vascular dysfunction and the response to interventions may be gained by examining the response to a sub maximal dose of NTG or a series of NTG doses. The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is under explored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature.

CONCLUSION

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1) Endothelial dysfunction is present in SLE patients even in the absence of traditional cardiovascular risk factors.

2) FMD using vascular ultrasonography on brachial artery represents a non-invasive, reproducible and cost effective tool for the assessment of endothelial dysfunction.

3) Impaired flow mediated dilatation of brachial artery may be an early physiological feature of endothelial dysfunction and may precede the anatomical increase in carotid intimal thickness.

4) FMD of brachial artery has significant negative correlation with disease activity and antidsDNA antibody.

5) Future prospects of FMD technique include

   a) early screening module for endothelial dysfunction and to start on drugs with pleotrophic effect like statins, ACE inhibitors and Aspirin.

   b) serial screening method to ascertain improvement while on treatment with immunosuppressive agents and vasodilators.

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BIBLIOGRAPHY

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APPENDICES

ABBREVIATIONS

SLE	Systemic lupus erythematosus
FMD	Flow-mediated vasodilatation
CVD	Cardiovascular disease
IMT	Intima-media thickness
LAI	Lupus activity index
EDD	Endothelial dependent vasodilatation
BMI	Body mass index
SLEDAI	SLE Disease Activity Index
SLICC	Systemic Lupus International Collaborating Clinics
ESR	Erythrocyte sedimentation rate
CRP	C Reactive protein
ANA	Anti nuclear antibody
Anti dsDNA	Anti double stranded DNA
aCL	Anticardiolipin antibody

PROFORMA

NAME: **AGE/SEX:** **OP/ IP No:**

RCC No :

ADDRESS: **OCCUPATION:**

H/O PRESENT ILLNESS: **TOTAL DURATION OF ILLNESS**

Fever	Malaise	Fatigue
Malar rash	Discoid lesion	Oral ulcer
Alopecia	Photosensitivity	
Purpura	Raynaud's	Gangrene
Joint Symptoms		
Myalgia	Weakness	Headache
Visual sym	Mood	Seizures
Chest pain	Palpitation	Dyspnoea
Syncope	Pedal edema	
Cough	Expectoration	Hemoptysis
Hematuria	Oliguria	Facial puffiness

OTHERS

PAST HISTORY:

PERSONAL HISTORY:

TREATMENT HISTORY:

PHYSICAL EXAMINATION:

Fever Anaemia clubbing cyanosis LN PE JVP

MUCOCUTANEOUS

OTHERS

PULSE BP RR
CVS RS ABDOMEN

CNS Fundus: MSS:

INVESTIGATIONS:

Hb TC DC ESR 1 hr Platelets
BT CT PT INR APTT
Urea Cr Uric acid Sugar T.Bilirubin
ALT AST
SAP LDH CPK Na K HCO 3 Cl
Lipid profile
Urine R/E PCR 24 hrs protein
ANA Anti dsDNA ACL
LAC VDRL CRP
Complement ENA

Renal Biopsy :

ECG CXR PA View
ECHO Carotid IMT

Flow Mediated Dilatation **Baseline** **FMD**

ASSESSMENT SLEDAI SLICC

MANAGEMENT

NSAIDS STEROIDS Pulse
Oral
IMMUNOSUPPRESSANTS

ANTICOAGULANTS/ANTIPLATELETS

SLEDAI

Systemic Lupus Erythematosus Disease Activity Index

Descriptor	Definition	Weighted Score
Seizure	Recent onset; exclude metabolic, infectious, or drug-related causes	8
Psychosis	Altered ability to function in normal activity owing to severe disturbance in the perception of reality; includes hallucinations, incoherence marked by loose associations, impoverished thought content, marked illogical thinking, and bizarre disorganized or catatonic behavior; exclude the presence of uremia and offending drugs	8
Organic brain syndrome	Altered mental function with impaired orientation or impaired memory or other intellectual function, with rapid onset and fluctuating clinical features; includes clouding of consciousness with reduced capacity to focus and inability to sustain attention on environment, and at least two of the following—perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, and increased or decreased psychomotor activity; exclude metabolic infectious and drug-related causes	8
Visual	Retinal changes from systemic lupus erythematosus cytoid bodies, retinal hemorrhages, serous exudate or hemorrhage in choroid, optic neuritis (not due to hypertension, drugs, or infection)	8
Cranial nerve	New onset of sensory or motor neuropathy involving a cranial nerve	8
Lupus headache	Severe, persistent headache; may be migrainous, unresponsive to narcotic analgesia	8
Cerebrovascular accident	New syndrome; exclude arteriosclerosis	8
Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages; vasculitis confirmed by biopsy or angiogram	8
Arthritis	More than two joints with pain and signs of inflammation (tenderness, swelling, or effusions)	4

Descriptor	Definition	Weighted Score
Myositis	Proximal muscle aching or weakness associated with elevated creatine phosphokinase/aldolase levels, electromyographic changes, or biopsy specimen showing myositis	4
Casts	Heme, granular, or erythrocyte	4
Hematuria	>5 erythrocytes per high-power field; exclude other causes (stone, infection)	4
Proteinuria	>0.5 g of urinary protein excreted per 24 hr; new onset or recent increase of >0.5 g/24 hr	4
Pyuria	>5 leukocytes per high-power field; exclude infection	4
New malar rash	New onset or recurrence of inflammatory type of rash	4
Alopecia	New or recurrent; patch of abnormal, diffuse hair loss	4
Mucous membrane	New onset or recurrence of oral or nasal ulceration	4
Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening	4
Pericarditis	Pericardial pain with at least one rub or effusion; confirmation by ECG or echocardiography	4
Low complement	Decrease in CH50, C3, or C4 levels (to less than the lower limit of the laboratory-determined normal range)	2
Increased DNA binding	>25% binding by Farr assay (to more than the upper limit of the laboratory-determined normal range, e.g., 25%)	2
Fever	>38°C after exclusion of infection	1
Thrombocytopenia	<100,000 platelets	1
Leukopenia	Leukocyte count <3000/mm^3 (not due to drugs)	1

SLICC
Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for Systemic Lupus Erythematosus

Item	Score
Ocular (either eye by clinical assessment)	
Any cataract ever	0, 1
Retinal change or optic atrophy	0, 1
Neuropsychiatric	
Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) or major psychosis	0, 1
Seizures requiring therapy for 6 mo	0, 1
Cerebrovascular accident ever (score 2 if >1)	0, 1, 2
Cranial or peripheral neuropathy (excluding optic)	0, 1
Transverse myelitis	0, 1
Renal	
Estimated or measured glomerular filtration rate <50%	0, 1
Proteinuria >3.5 g/24 hr	0, 1
or End-stage renal disease (regardless of dialysis or transplantation)	*or* 3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P_2)	0, 1
Pulmonary fibrosis (physical and radiographic)	0, 1
Shrinking lung (radiograph)	0, 1
Pleural fibrosis (radiograph)	0, 1
Pulmonary infarction (radiograph)	0, 1
Cardiovascular	
Angina or coronary artery bypass	0, 1
Myocardial infarction ever (score 2 if >1)	0, 1, 2
Cardiomyopathy (ventricular dysfunction)	0, 1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	0, 1
Pericarditis for 6 mo or pericardiectomy	0, 1
Peripheral vascular	
Claudication for 6 mo	0, 1
Minor tissue loss (pulp space)	0, 1

Significant tissue loss ever (e.g., loss of digit or limb) (score 2 if >1 site)	0, 1, 2
Venous thrombosis with swelling, ulceration, or venous stasis	0, 1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver or gallbladder ever, for any cause (score 2 if >1 site)	0, 1, 2
Mesenteric insufficiency	0, 1
Chronic peritonitis	0, 1
Stricture or upper gastrointestinal tract surgery ever	0, 1
Chronic pancreatitis	0, 1
Musculoskeletal	
Muscle atrophy or weakness	0, 1
Deforming or erosive arthritis (including reversible deformities, excluding avascular necrosis)	0, 1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	0, 1
Avascular necrosis (score 2 if >1)	0, 1, 2
Osteomyelitis	0, 1
Tendon rupture	0, 1
Skin	
Scarring chronic alopecia	0, 1
Extensive scarring of panniculus other than sculp and pulp space	0, 1
Skin ulceration (excluding thrombosis for >6 mo)	0, 1
Premature gonadal failure	0, 1
Diabetes (regardless of treatment)	0, 1
Malignancy (exclude dysplasia) (score 2 if >1 site)	

CONSENT FORM

PATIENT CONSENT FORM

STUDY TITLE
ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSIS

Study Centre : Department of Rheumatology,
Madras Medical College, Chennai – 600 003

Patient's Name :
Patient's Age :
Identification Number : Patient may check (✓) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethics committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and to faithfully co-operate with the study team, and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ☐

I hereby consent to participate in this study on **ENDOTHELIAL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSIS** ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological and urine examination. ☐

Signature / Thumb Impression _____ Place _____ Date _____

Patient's Name and Address: _____

Signature of the Investigator : _____ Place _____ Date _____

Study Investigator's Name : _____

ETHICAL COMMITTEE APPROVAL

INSTITUTIONAL ETHICAL COMMITTEE
GOVERNMENT GENERAL HOSPITAL & MADRAS MEDICAL COLLEGE,
CHENNAI-600 003.

Telephone: 044-2530 5000
Fax : 044 – 25305113

K.Dis.No. 0029094/P & D3/Ethies/Dean/GGH/09 Dated: 10/2/2009

Title of the work : "Endothelial dysfunction in systemic Lupus Erythematosis"

Principal Investigator : Dr. S. Rajesh M.D.,

Department : Rheumatology, MMC, ch-3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on . 17 – 2 – 2009 at 2 P.M in Government General Hospital, Deans, Chamber, Chennai-3.

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The principal investigator and their term are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate form the area of the work for which I applied for ethical clearance
5. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
6. You should abide to the rules and regulations of the institution(s)
7. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

SECRETARY
IEC, GGH,CHENNAI

CHAIRMAN
IEC, GGH,CHENNAI

DEAN
GGH &MMC, CHENNAI

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